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PEOPLE AND EVENTS

B. C. Johnson Recipient of Award

B. C. JOHNSON, the recipient of the 1960 American Feed Manufacturers' Association Award, has contributed much to basic and fundamental knowledge of nutrition.

During the years of 1957, 1958, and 1959 Dr. Johnson was the author or co-author of 37 scientific papers published in eight scientific journals in this country and several foreign journals. Dr. Johnson has been very versatile in his research efforts, having studied the nutritional status of many species of animals; he has been active in many specialized areas of nutrition and biochemistry and his research findings have contributed much to our present knowledge of the nutrition and physiology of animals, including man. His interests have embraced studies of nitrogen sources for sheep, vitamin deficiencies of calves, vitamin B_{12} metabolism and function, metabolism of radioactive vitamin A, radiation sterilization of foods, nutritional role of antioxidants and other chemical compounds, effect of antibiotics on the intestinal synthesis of vitamins, and amino acid metabolism and requirements of various species of animals.

B. C. Johnson

Dr. Johnson was born in Regina, Saskatchewan, Canada, April 28, 1911. He was graduated with the B.A. degree in organic chemistry from McMaster University, Ontario, Canada, in 1933 and 1 yr. later was awarded the M.A. degree by that institution. During the following 3 yr. he was employed by Dominion Canner's Ltd. at Hamilton, Ontario.

In 1940, the University of Wisconsin awarded him the Ph.D. degree in biochemistry. From 1940 to 1942 he served as a postdoctoral fellow in the Department of Animal Science at the University of Illinois and subsequently took a position for 1 yr. as a research biochemist with the Golden State Co., Ltd., in San Francisco, California. In 1942 he returned to the University of Illinois and has been a member of the staff of the Department of Animal Science since that date.

J. A. Nelson Honored

Recognizing the need for putting dairy science into practice, in 1933 Montana State College inaugurated Montana Dairy Industry Week, an annual course of instruction for plant producers and milk producers. This year's program, which carried out the theme of Processing, Promotion, and Pricing, was dedicated to J. A. NELSON in recognition of the great benefits of this instruction to the dairy industry.

As a token of appreciation of his long, continuous, and devoted service to the dairy industry, he was presented with a large bound volume entitled In Tribute to Dr. John A. Nelson. This volume contained a considerable number of congratulatory letters, telegrams, and messages sent him from his many friends and co-workers in Montana and throughout the United States and Canada. In addition, he was presented with a large portrait to be placed in the department in his memory. A liberal cash honorarium accompanied the dedication, and the Nelson home was presented with a large bouquet of mammoth red roses.

J. A. Nelson

The Dairy Science Club, a student organization which he organized and sponsored, awarded him a plaque, a certificate for eminent service to the dairy industry, and named him Outstanding Dairyman for 1960.

G. M. TROUT of Michigan State University, M. J. FRAMBERGER of the American Dairy Association, DEAN P. C. GAINES of M.S.C., and two former students, FERGUS MITCHELL and KENNETH MCIVER, spoke on various phases of Dr. Nelson's long service to the Dairy Industry in the state and the nation. This celebration was attended by 200 people.

Dr. Nelson went to Montana State College in 1922, as an instructor. At that time the Dairy Department was a subsidiary of the Animal Husbandry Department. His rise in rank was rapid, being elevated to the rank of professor in 1929, at which time he was appointed head of the newly created Department of Dairy Industry, which involved dairy manufacturing teaching and extension. In 1938, the department was enlarged by the addition of dairy production and the innovation of research in dairy products and in dairy production.

In 1948, he was appointed as the first Graduate Dean, a position he held along with his executive duties in the department. As Graduate Dean, he organized the first graduate faculty and greatly increased the graduate offerings on the Master's level. When he relinquished that position in 1955, work toward the Ph.D. degree was offered in several departments.

In 1956, due to the age limit and at his request, he was relieved from the department head duties and continued as a teaching and research professor, the position he still holds.

Along with his executive duties, Dr. Nelson found time to take an active part in research. He is author or co-author of numerous bulletins, circulars, pamphlets, and magazine articles, and co-author of a text on Judging Dairy Products, a field in which he has much interest and influence. His services as speaker, consultant, and dairy products judge at dairy short courses, conventions, and dairy shows were in demand. He will be long remembered by his students as an inspiring and dynamic teacher and always helped them with their problems.

Dr. Nelson gained recognition in many honorary societies and scientific organizations. He is a member of Sigma Xi, Phi Kappa Phi, Gamma Sigma Delta, Alpha Zeta, AAAS, Montana Academy of Science, Dairy Shrine Club, and has been a life-long member of A.D.S.A., serving the Association on numerous committees, chairman of the Western Section, as director, and as president.

He received the A.D.S.A. Award of Honor in 1959. He is listed in American Men of Science, Leaders in American Science, Who Is Who in American Education, Who Is Who in the West, and Who Is Who in America.

In addition to his professional duties, he has found time for constructive work in his community, serving faithfully in the Methodist church, on the local hospital board, and took part in many fund-raising campaigns for the improvement of the community in which he lives.

A. & T. Agricultural Leader Receives Top Southern Award

An extension dairy specialist at the Agricultural and Technical College in Greensboro, North Carolina, was honored as the 1960 winner of the Professional Agricultural Workers Service Award.

Winner of the award is R. L. (Bob) Wynn, who has spent 15 yr. helping farm families in North Carolina raise their level of living by developing dairy enterprises. His selection was made by a committee of the U. S. Department of Agriculture. Agricultural workers in 14 southern states were considered for the honor.

R. L. Wynn

At the time of his appointment in 1945, Wynn was the first Negro agricultural specialist to work for a land-grant college in the United States. He still is the only dairy specialist. Since 1945 he has helped 60 farmers establish Grade A dairies and another 900 farmers start production of manufacturing milk. These dairymen have an estimated capitalization of $18 million and a gross annual income of $4.4 million.

Most of the dairies have been an outgrowth of Wynn's work with young people. Under his leadership, about 1,500 boys and girls are carrying dairy projects. Each year he holds from eight to ten area or district junior dairy shows and assists with about 15 county shows. In 1955, he organized an annual 4-H Club dairy cattle judging contest. More recently, he has helped start a state 4-H Club dairy calf chain program. To help adult farmers, he organized an annual dairy production short course at A. & T. College in 1948. Over 1,000 farmers have attended.

Prior to joining the staff at A. & T. College, Wynn established the present school of agriculture at Fort Valley, Georgia. Before that he was a teacher of vocational agriculture at Forsyth and Dixie, Georgia.

Wynn, a native of Taylor County, Ga., earned a B.S. degree from Hampton Institute and a M.S. degree from Michigan State University. He is a deacon at Providence Baptist Church in Greensboro, a master Mason, an active PTA supporter, and member of the American Dairy Science Association.

The agricultural extension program at A. & T. College is part of the N. C. Agricultural Extension Service, which includes specialists at A. & T. College and N. C. State College.

Conference at the University of Arizona

The Tenth Annual Dairy Industry Conference sponsored by The University of Arizona was held January 11 and 12, 1961, at Del Webb's Hiway House Hotel. Speakers included F. E. Nelson, who had joined the staff at Arizona coming from Iowa State University, H. S. Christiansen, Carnation Co., Los Angeles, G. R. Wood, Food and Drug Administration, Los Angeles, and R. P. Harris, Scholle Container.

Dairy Students at University of Georgia Receive Scholarships

Nineteen students in the Dairy Department of the University of Georgia were awarded scholarships of $200 to $300 at the recent annual fall banquet of the Dairy Science Club. With the exception of the $300 Borden Foundation Award, all of the scholarships were made available by interested dairy companies and organizations and individuals in Georgia. This is the largest number of awards ever made in the Dairy Department.

In making the awards, H. B. Henderson, Head of the Dairy Department, praised the dairy industry of the State for its interest in

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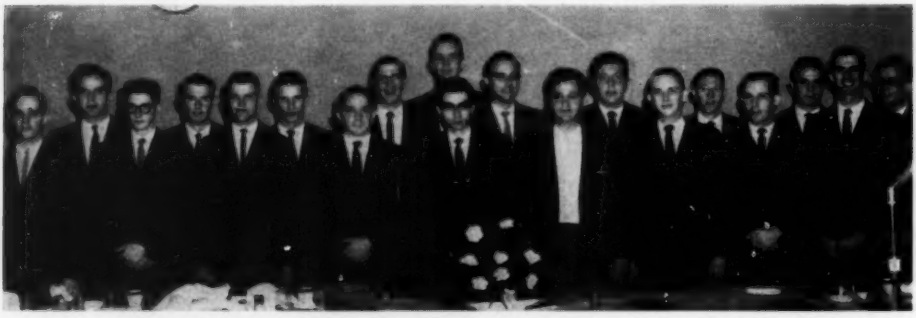
the future welfare of the industry by offering encouragement to young men of outstanding ability who are preparing for a vocation in this industry. He emphasized that the scholarships meant much more than financial assistance to those students meeting the requirements set by the scholarship committee. "These awards have given confidence to all students in the Department," he said. "They know they are entering a profession in which leaders are looking beyond the present, and attempting to attract the most capable personnel available, to the end that continued progress may be made."



Left to right: Dan Barrett, Calhoun, Ga.; Joseph Harby, Walton, N. Y.; Elwin Rivenburg, Maine, N.Y.; Melvin Durham, Union Point, Ga.; Curtis Tumlin, Madison, Ga.; George Adams, Wrens, Ga.; Ronald Waddell, Sparta, Ga.; Ronald Jones, Pleasant Valley, N.Y.; Charles Jantzen, Cambridge Heights, N.Y. (rear); Rudolfo Villavicencio, Guatemala; Thos. R. Norris, New York City; Albert Dattell, Queens, N.Y.; John Dye, Middleton, Ga.; Johnny Stone, Hoagansville, Ga.; James Smith, Augusta, Ga.; Wesley Blair, Boston, Ga.; Joe Shellnutt, Lovejoy, Ga.; Zack Seymour, Bowman, Ga.; George Norman, Washington, Ga.

Former students in the Dairy Department at the University of Georgia are beginning to make their contribution to the scholarship program. FRANK WIGHAM, manager and owner of Southland Dairy Products Company, Bainbridge, Georgia, was listed among the scholarship contributors this year. GEORGE TWOROGER, manager and owner of Expert Dairy Service, Miami, Florida, recently made a substantial gift to the University to be used to provide scholarships to dairy students.

RALPH HODGSON, Chief of the Animal Industry Division of the Agricultural Research Service, Beltsville, Maryland, spoke at the banquet at which the awards were made. Dr. Hodgson is president of the American Dairy Science Association.

Illinois News

Milk Concentrates Conference

The 5th Milk Concentrates Conference will be held at the University of Illinois on October 24 and 25, 1961.

This conference was first sponsored by the Eastern Utilization Research Branch of the United States Department of Agriculture at Philadelphia, Pennsylvania. P. A. WELLS, Director of this laboratory and the Directors of a number of Experiment Stations in the East, planned the first and second conferences which were held at the USDA laboratory in Philadelphia. The third concentrates conference was held at the University of Wisconsin in October, 1958, and the fourth one was held at Michigan State University in March, 1960.

Plans are being made to develop a program that will be pointed toward problems that confront the industry. Leaders in the dairy industry and in the experiment stations will be invited to suggest topics for consideration. The Department of Food Technology will be the host.

Iowa State News

J. T. HUBER joined the staff of Virginia Polytechnic Institute, Blacksburg, Virginia, as Assistant Professor in the Department of Dairy Science in October, 1960. Dr. Huber received the doctorate in dairy nutrition at Iowa State University in 1960, with his thesis titled Digestion and Absorption of Carbohydrates Delivered Directly to the Bovine Omaso-Abomasum.

Earlier in the year, D. K. HOTCHKISS, also a recent Ph.D. graduate of Iowa State University in Dairy Nutrition, assumed the position of Manager of Research Statistics Department with Ralston Purina Company, St. Louis, Missouri.

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of Statistics at Iowa State University for the 11-wk. period from June 5 through August 18, 1961. The Departments of Statistics of three other universities, Kansas State, Utah State, and the University of Wyoming, are cooperating with Iowa State's statistical center in presenting this institute.

Financial support in the form of stipends, dependency allowances, and travel allowances will be awarded to 50 eligible applicants. All American college and university teachers who are, or who during the 1961-62 academic year will be, required to teach one or more courses in statistics as part of their regular assignments are eligible for consideration.

The institute is planned to provide additional basic training in statistics for present and prospective teachers who, though well-grounded in other fields, have limited backgrounds in statistics. Also, it will provide more advanced courses and seminars designed to keep college and university teachers abreast of new developments.

Courses are scheduled in Statistical Methods, Theory of Statistics, Experimental Design, Survey Designs, Topics in Foundations of Probability and Statistics, and Intermediate Applied Decision Theory. In addition, an opportunity will be provided for those interested to observe a demonstration class in Principles of Statistics at the undergraduate level. The faculty will include the institute director, T. A. BANCROFT, director of the Iowa State University Statistical Laboratory and head, Department of Statistics; R. J. BUEHLER, associate professor of statistics, Iowa State University; H. T. DAVID, associate professor of statistics, Iowa State University; H. C. FRYER, head of the Department of Statistics and Statistical Laboratory director, Kansas State University; H. O. HARTLEY, professor of statistics, Iowa State University; the institute associate director, D. V. HUNTSBERGER, associate professor of statistics, Iowa State University; and R. L. HURST, head of the Department of Applied Statistics and Statistical Laboratory director, Utah State University. Guest lecturers will present a series of special seminars.

Requests for information or application forms should be addressed to: The Director, Summer Institute in Statistics, 102 Service Building, Iowa State University, Ames, Iowa.

Short Courses at Michigan State University

The short course schedule for 1961 at Michigan State University, East Lansing, is as follows:

Quality Control and Analysis—Jan. 9-20; Cottage Cheese and Cultures—Jan. 23-Feb. 3; Market Milk—Feb. 6-17; Ice Cream—Feb. 20-Mar. 3; Dairy Plant Supervision—Mar. 6-17; Dairy Engineering—Feb. 28-Mar.1.

Anyone interested should write to Short Course Department, Michigan State University, East Lansing, Michigan.

Ohio News

Plans currently are being completed for the 28th Annual Dairy Technology Conference, to be held on the Ohio State Campus, February 7-9, 1961. The Conference will cover five major areas: Milk Supply; Plant Management and Operation; Engineering; Quality Control and Cultured Products; and Ice Cream Operations. Approximately 500 are expected to attend the various concurrent sessions, which will include presentations by some 35 visiting speakers and several staff members.

The College of Agriculture and Home Economics is holding a series of meetings with Agricultural Industry leaders to acquaint them with the Agribusiness and Agriscience aspects of the revised curricula offered by the College. Under the leadership of DEAN R. M. KOTTMAN, the program is designed to enlist the support of key industrial personnel in interesting more students in the varied opportunities which exist in the broad field of Agriculture.

As another aspect of the College's efforts to provide prospective students with every possible opportunity to choose a career in Agriculture best suited to their interests and qualifications, three Career Days are held during the academic year. On these days, high school students and their parents and teachers from throughout Ohio are invited to the College, where the broad aspect of Agriculture careers is discussed with them. By means of literature, displays, and department tours, the individual departments then present to those interested the programs and opportunities available in specific fields. The first of these events was held on December 3, 1960, with about 200 in attendance. The second is planned for February 4.

I. A. GOULD, Chairman of the Dairy Technology Department, returned November 1 from 3 mo. association with United Nations International Childrens Emergency Fund and the Foreign Agricultural Organization of the United Nations. During this association, he conducted a survey of dairy training needs and opportunities abroad, with particular reference to India. Countries visited in connection with the program included England, Italy, Yugoslavia, Iran, Iraq, Pakistan, and India. The purpose of the survey was to determine ways and means of providing ample qualified personnel to operate the dairy schemes being developed in those countries.

W. J. HARPER, who has been on leave from the University and the Ohio Agricultural Ex-

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CONTENTS

SECTION I states the objectives and requirements of the program.

SECTION II contains training schedules for all phases of plant operation for all types of plants. Many questions are asked for the trainee to answer before passing from one phase of training to the next, and progress reports and rating forms are provided.

SECTION III covers management development. Many study projects are outlined which are not only essential to management development but may result in savings to the plant that will more than pay the cost of training.

The Appendix contains a list of professional and trade organizations and reference reading publications.

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periment Station for 1 yr., returned to the Dairy Technology Department January 1. During his absence, the major portion of his time was devoted to studies at the Sweden Dairy Research Institute, Alnarp, Sweden. He also studied research developments at various dairy research centers in Northern Europe.

Two new research projects recently were initiated in the Department of Dairy Technology. A study of the flavor of Cheddar Cheese, supported by a contract with the Dairy Section, Eastern Agricultural Utilization Branch, United States Department of Agriculture, and administered through the Ohio Agricultural Experiment Station, was activated on November 1, 1960. A project dealing with factors affecting quality of consumer packages of cheese, supported by the American Dairy Association, was activated on January 1, 1961.

R. V. RAO returned to his native India on December 15, 1960, after studying in the Department of Dairy Technology for the past year.

P. S. RAO, of India, and R. J. RICHARDS, Australia, received Ph.D. degrees in December and are returning to their respective countries.

H. E. KENNEDY, Assistant Professor of Dairy Technology and Research Associate, Ohio State University Research Foundation, has resigned his position to become Director of Dairy Product Research with Johnson and Johnson Co., New Brunswick, New Jersey.

Marschall Laboratory Appoints Roundy

Z. D. ROUNDY has been named to the staff of the newly formed Laboratory Testing Division of Marschall Dairy Laboratory, Inc. He will be available to the cheese industry for consultation on all types of production and quality control problems.

Dr. Roundy comes to Marschall Laboratories from the Research Division of Armour and Company, Chicago, where he spent the last 23 yr. His B.S. and M.S. degrees are from Utah State Agricultural College, Logan, Utah, and his Ph.D. in Dairy Industry from the University of Wisconsin.

Z. D. Roundy

Dr. Roundy is widely known in the industry for his work in the development of the hydrogen peroxide–catalase treatment of milk for the manufacture of cheese. He obtained the original patent for manufacture of Swiss Cheese by the hydrogen peroxide–catalase system. He is experienced in all fields of the dairy industry and has done research and development work on the enzymatic treatment of milk, the enzymatic treatment of cheese to aid flavor development, production of all types of hard and soft cheese, of process cheese and development of new processes in cheese manufacture. He also has had commercial experience in the manufacture of butter, ice cream, and evaporated milk.

For a number of years, Dr. Roundy has been active in the Research Committee of the National Cheese Institute. He has been on the Subcommittee on Sanitary Standards of the Dairy Industry Committee, and the Dairy-wide Co-ordinating Committee on Nutritional Research, as well as on the Cheese Advisory Committee to the Quartermaster Food & Container Institute. He is a member of the American Dairy Science Association and has served on several committees in this organization.

STUDENT NEWS SECTION

J. E. JOHNSTON, Editor

A Section Devoted To News Of Student Activities

CHAPTER ACTIVITY CONTEST

The student chapter activity contest is to be held again during the 1961 Convention. The chapter activity is evaluated by a panel of judges who base their selection on the chapter exhibit (Scrapbook or Annual) and on the chapter annual report. Information on both these activities was mailed to each chapter early in December. Entry in the contest can be assured by having the annual report in on time and by bringing an exhibit to the Convention. The outstanding chapter receives a certificate and a $100 award. Runners-up receive certificates of merit. Now is the time to start planning your chapter participation in this contest.

GRADUATE STUDENT SCIENTIFIC PAPER PRESENTATION CONTESTS

The Scientific Paper Presentation Contests will be conducted again during the 1961 Convention in both the Production and Manufacturing sections. Each institution is entitled to enter one participant in each contest. Participants must be student affiliate members and must present a paper in conformity with the rules and regulations of the appropriate section of the Association. Entry in the con-

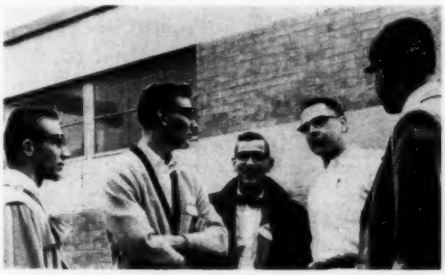
test is accomplished by addressing a letter to the section chairman indicating desire to enter the contest. This letter must be countersigned by the Major Professor and Department Chairman and should accompany abstracts of the paper as specified in the Call for Papers for the 1961 Annual Meeting as published in the JOURNAL OF DAIRY SCIENCE, Vol. 43, page 1542 (October, 1960). A carbon copy of the letter of entry and four additional copies of the abstract should be mailed to one of the following:

A. For Production Section Award
Dr. Louis Boyd
Dairy Department, Univ. of Tennessee
Knoxville, Tennessee

B. For Manufacturing Section Award
Dr. W. F. Shipe
Department of Dairy Industry
Cornell University
Ithaca, New York

Contestants will be judged by a panel of judges in each section and the first-place contestant will receive a certificate and a $100 prize. Runners-up will receive Certificates of Merit. The deadline for submission of papers is February 26, 1961.

DAIRY CLUB ANNUAL FALL FIELD TRIP



Warren Suchovsky, Steve Crotz, Dr. Roy Emery (Club Advisor), and Pete Pierpont discuss production of pharmaceutical products with an Upjohn Fermentation Plant Supervisor.

On November 5, the Michigan State University Dairy Club held its annual fall field trip. Eleven Dairy Club members and the club advisor, ROY EMERY, visited the Upjohn Pharmaceutical Company at Kalamazoo, Michigan.

With ROLLAND GESSERT as guide, the group first toured the downtown research tower. Here were viewed many of the facilities which are used for research purposes by the nearly

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600 people engaged in research and development at Upjohn.

Then, the group traveled to the Portage road manufacturing plant. There they toured the fermentation plant, where they saw the machinery and production processes used in the production of many pharmaceutical products.

They then toured the main manufacturing building which has 33 acres of floor space under one roof. More than 600 Upjohn products are packaged here for shipment to many different parts of the world.

The last stop on the tour was to the research farm, where the group saw the many facilities available for the Veterinary Research Program.

Following the tour, the group had lunch as guests of the Upjohn Company at Gull Lake Inn.

FROM THE SECRETARY'S DESK

Fellow members, have you ever said to yourself, "Why doesn't the Editor publish this?", or, "Why does he publish that in the JOURNAL?", or, "Why doesn't the President do this or that?", or, "What does the Secretary do with his time?", or, "Why did the Board of Officers vote to do it that way?" If some of these questions never come to your mind, then you are not a "thinking member with a dairyman's taste for curiosity."

If you have a gripe or something good to say about something or somebody, it doesn't pay to harbor it too long. Letters to the Editor or any one of the Officers, same to be published in the JOURNAL if it seems feasible, are always in order and I am sure will receive a courteous reply.

—H. F. JUDKINS

News of Borden's

The Borden Company has purchased a majority interest in Pasteurizadora de Helados Club, S.A., a leading manufacturer and distributor of ice cream in Venezuela.

Helados Club was organized in 1954, the result of a merger of two companies. Its headquarters and principal plant are in Caracas, the Venezuelan capital, and a second plant is located in Maracaibo. With factories in the two principal cities, Helados Club extends its distribution to Barquisimeto, the third largest center, Maracay, Ciudad Bolivar, and other cities. The Venezuelan management will continue to operate the business, aided by Borden technical personnel.

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4. Conduct research on new ideas suggested by you.

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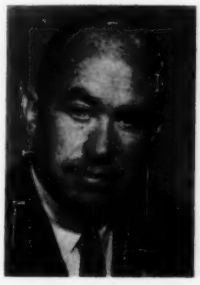
Western Condensing Promotes Alesch

The final step in completing the modernization and expansion program for the Technical Service Department at Western Condensing Company, Division of Foremost Dairies, was recently taken with official announcement that E. A. ALESCH has been promoted to the post of Technical Service Manager.



E. A. Alesch

Mr. Alesch has been with Western since 1947 in product development, technical service, and sales capacities. He has been associated with the dairy and baking industries for 25 yr. and is active in the Dunwoody Institute and the American Institute of Baking.

News from the National Institutes of Health

The appointment of D. R. LINDSAY as Chief of the Division of Research Grants has been announced by the National Institutes of Health, Bethesda, Maryland. He is succeeding E. M. ALLEN, who has been brought into the Office of the Director, NIH, as Associate Director for Research Grants. Dr. Lindsay will be assisted by C. C. POWELL, who has been named Deputy Chief.

Dr. Lindsay is a career Public Health Service Officer, who came to the Division of Research Grants in 1953. In 1955, he was made Scientific Director of the Division and in 1957, Assistant Chief. Prior to 1953, he was associated with the Communicable Disease Center in the Bureau of State Services.

Dr. Powell is also a career Public Health Service Officer previously identified in the field of radiological health in several Service capacities. Most recently, he has been Assistant Grants Branch Chief for Clinical Research in the Grants Review Branch of the Division.

Ralston Purina Company Research Fellowship Awards for 1961-62

The objective of these fellowships is to assist in the training of additional personnel for leadership in the science of livestock and poultry production.

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sonal qualifications and submits a completed application may be eligible. The application and required information must be in the hands of the Awards Committee by March 1 of the year the Award is to be made.

Not more than ten Research Fellowships shall be awarded annually, based upon qualifications, as follows: three each in dairy husbandry, animal husbandry, and poultry husbandry; and one in the field of veterinary science.

Amount of Fellowship—$2000.

Application blanks for these Fellowship Awards may be obtained by writing the Ralston Purina Research Awards Committee, c/o Mr. J. D. Sykes, Ralston Purina Company, St. Louis 2, Missouri.

Completed Theses

Ph.D. Degree:

P. S. Rao—Isolation and characterization of the heat-inducted protein complex in milk. The Ohio State University, Columbus.

Ross J. Richards—The effects of antibiotics on lactic streptococci and lactic streptococcal host-phage relationships. The Ohio State University, Columbus.

Dairy Technology Societies

Atlanta—A Christmas party was given at Yohannin's Restaurant December 5. Special entertainment was provided and the speaker was Don Stewart, Announcer WSB-TV.

Central Illinois—This group also enjoyed a Christmas party—at the University YMCA, Champaign. The speaker was G. W. Shadwick, director of foreign operations for Beatrice Foods Company. His topic was A Milkman in Malaya, illustrated with colored movies.

Detroit—The annual Ladies Night and Christmas party was held at Cregars on Grand River, December 12. Speaker was Hap Bowser of Dairy Pak.

Kansas City—A buffet turkey dinner was served at the Elks Club for the December (Christmas) meeting. No speeches or formal program were provided, but the evening was entirely social.

Ohio—The January meetings—held January 4 for the Northeastern Ohio group and January 5 for the Maumee Valley Society—featured Mike Hales of the Klenzade Company, speaking on How Should Cultures and Cultured Products Be Handled in the Dairy Plant? January meeting of the Cincinnati and Central Ohio Societies was held January 4 and 5, respectively. Wayne Churchill of the American Dairy Association discussed Sales and Sales Promotion.

Philadelphia—December meeting was held December 8, with Ridgeway Kennedy, Jr., Chairman of the Board of Abbotts Dairies, as speaker. Mr. Kennedy addressed the group as Chairman of the Standards Committee of the International Association of Ice Cream Manufacturers, providing the latest information from the Federal Food & Drug Administration.

Tri-State—A buffet dinner-dance was given December 14 at the Mona Lisa Lounge—the group's usual Christmas event.

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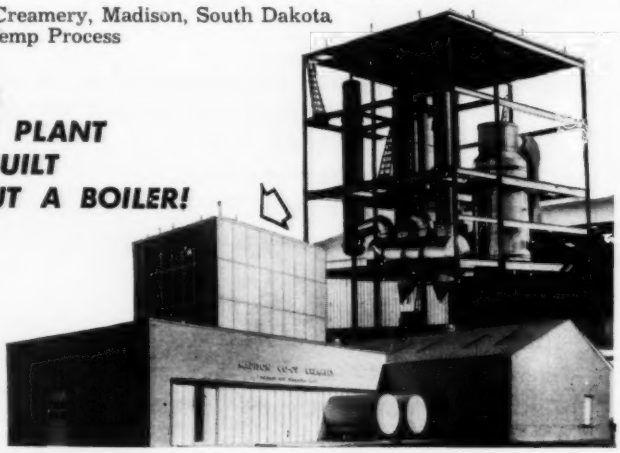

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RESEARCH PAPERS

VOLATILE CARBONYL COMPOUNDS IN STORED DRY WHOLE MILK[1]

OWEN W. PARKS[2] AND STUART PATTON

Department of Dairy Science, The Pennsylvania Agricultural Experiment Station, University Park

SUMMARY

Flavor constituents in the low-temperature vacuum distillate of reconstituted dry whole milks were largely carbonyl in nature. The 2,4-dinitrophenylhydrazone derivatives identified by paper and column chromatography, ultraviolet studies, and melting points revealed qualitative differences in the milks studied. The following were conclusively or tentatively identified in the distillate from an average dry whole milk: C_3 through C_7, C_9, C_{11}, and C_{15} n-alk-2-ones; C_1, C_2, and C_9 alkanals and *cis-* and *trans-*furfural. In contrast, a badly deteriorated powder yielded: C_3, C_4, C_6, C_{11}, and C_{15} n-alk-2-ones; C_1 through C_3, C_5 through C_7, C_9, C_{10}, and C_{12} n-alkanals; benzaldehyde, two mono- and two diunsaturated carbonyls. The poor powder could be distinguished from the average dry milk mainly by the large number and concentrations of aldehydes it contained.

The relative amounts of individual carbonyls in four additional dry whole milks manufactured and stored under various conditions were determined. The results suggest that the complex problem of organoleptically characterizing stale and oxidized flavor deterioration in dry whole milk stems in part from the large numbers and variable quantitative relationships of the carbonyl compounds involved.

The absence of methyl ketones in dry whole milk prepared with deodorized milk fat suggests a potential means of increasing the storage life of the product. Ketone formation appears to be an important deteriorative mechanism in stored dairy products containing milk fat.

PART I. RECOVERY AND IDENTIFICATION OF CARBONYLS

The use of dry whole milk for beverage purposes has been hindered by its inadequate dispersibility in water and its tendency to develop off-flavors that become increasingly objectionable during storage. Although progress has been made in improving dispersibility, the flavor problem has not yielded appreciably to concerted applied research. The fact that prevention of fat oxidation, and associated oxidized flavor, does not satisfactorily solve this problem has been generally recognized. A so-called stale flavor seems to evolve in the best of dry whole milks. The earlier work of Whitney *et al.* (14) contains observations on such a defect. A need for additional fundamental information on this problem, particularly with reference to the flavor compounds involved, served as the impetus for this study.

EXPERIMENTAL PROCEDURE

Collection of volatiles. The dry whole milk was reconstituted one part of powder to five parts of distilled water. This product was vacuum-distilled for 3 hr. at 35-40° C. and 15-20 mm. Hg in a 5-gal. Pyrex carboy, according to the

Received for publication July 12, 1960.

[1] Authorized for publication as Paper No. 2467 on June 24, 1960, in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Present address: Dairy Products Laboratory, Eastern Utilization Research and Development Division, USDA, Washington 25, D. C.

procedure employed by Day *et al.* (5) and in a later study by Wong *et al.* (15). Preliminary observations on the distillate trapped by means of a wet ice and two dry ice–ethanol traps indicated that flavor and aroma in the trapped distillate were due mainly to carbonyl compounds. The odor of the distillate was dissipated upon the addition of 2,4-dinitrophenylhydrazine[3] reagent, but was not materially influenced by the addition of acid or base. On the basis of these results, the carbonyl compounds in a relatively good dry whole milk and a decidedly stale, oxidized product, as determined by an experienced taste panel, were investigated to determine the differences in their carbonyl components. Twenty-five pounds of each product was employed in this investigation, necessitating five individual vacuum distillations on each powder.

Preparation of DNP hydrazones. To the distillate from reconstituted dry whole milk was added an acid solution of DNP hydrazine reagent (2 g. of 2,4-dinitrophenylhydrazine per liter of 30% H_2SO_4). The distillate-reagent mixtures from all three traps were combined and held at 40° F. for seven days (2). The DNP hydrazones were extracted with carbonyl-free hexane and the extract dried with Na_2SO_4. The solution then was filtered and evaporated under vacuum to a volume suitable for column chromatographic separations.

Chromatographic separations of DNP hydrazones. Initial separation of the DNP hydrazones was accomplished by the column partition chromatography methods A or B of Day *et al.* (4). Despite the merits of these procedures, they lack the ability to separate mixtures of DNP hydrazones with the following formulas: Alk-2-one$_N$, alkanal$_{N+1}$, alk-2-enal$_{N+2}$, alk-2,4-dienal$_{N+4}$, where N is equal to the number of carbon atoms in the ketone. Therefore, when it was apparent that mixtures of DNP hydrazones were present in the bands separating on the partition column, pure derivatives were obtained in many cases by collecting the forepart of the fraction or numerous small fractions from each band. In certain instances, further separations of mixtures from the partition column were accomplished by the adsorption chromatography method of Gordon *et al.* (7).

During the latter stages of the investigation the paper chromatographic method of Gaddis and Ellis (6) was published, which method succeeds in the separation of the previously mentioned DNP hydrazone mixtures. The tentative identification of some higher molecular weight carbonyls was effected with their procedure.

Identification of DNP hydrazones. Tentative identifications of the DNP hydrazones were accomplished by comparing retention volumes of the unknown derivatives on the column partition chromatograms with those reported by Day *et al.* (4) for known derivatives. Rf values by paper chromatography also were used for this purpose, i.e., the method of Huelin (8) for carbonyls up to eight carbons in chain length and the method of Klein and de Jong (11) for carbonyls of higher molecular weight. Both procedures were used in conjunction with ultraviolet spectral analysis in 95% ethanol and in 0.25 N NaOH in ethanol according to the reports of Braude and Jones (1) and Jones *et al.*

[3] 2,4-Dinitrophenyl is abbreviated DNP throughout.

(9). The use of alcoholic NaOH was especially effective in differentiating between aldehyde and ketone DNP hydrazones. Conclusive identifications were obtained by melting point and mixed melting points with authentic DNP hydrazones on a Fischer micro-melting point apparatus.

RESULTS AND DISCUSSION

Identification data for carbonyl compounds from the good and deteriorated dry whole milks are given in Tables 1 and 2, respectively.

TABLE 1

Spectral and melting point data in the identification of DNP hydrazones of carbonyl compounds from a relatively good dry whole milk

DNP[a] hydrazone	Absorption maximum (mμ)		Melting point (° C.)		
	Observed	Reported	Observed	Authentic	Mixed
Cis-furfural	369	379	230–235	232	.....
Trans-furfural	375	386	.....	.....	.....
Formaldehyde	345	346	165	166	166
Acetaldehyde	356	356	157	157	157
Acetone	362	363	126.5	127	126.5
Butanone-2	360	362	105.5	116	106
Pentanone-2	362	362	143	143	143
Hexanone-2	360	362	106	106	106
Heptanone-2	362	362	71– 72	72	72
Nonanal	358	358	.....	102	.....
Nonanone-2	362	362	37	38	38
Undecanone-2	360	362	.....	.....	.....
Pentadecanone-2	360	362	.....	.....	.....

[a] Tentative identifications based on data from paper and column chromatograms.

TABLE 2

Spectral and melting point data in the identification of DNP hydrazones of carbonyl compounds from a deteriorated dry whole milk

DNP[a] hydrazone	Absorption maximum (mμ)		Melting point (° C.)		
	Observed	Reported	Observed	Authentic	Mixed
Formaldehyde	345	346	165	166	166
Benzaldehyde	378	378	237	238	238
Acetaldehyde	356	356	157	157	157
Acetone	362	363	126	127	126.5
Propanal	358	358	152	149–152	150–152
Butanone-2	360	362	116.5	116	114
Pentanal	358	358	98	99	98
Hexanal	356	358	106	106	106
Heptanal	358	358	106	104	104
Octanal	358	358	105	106	105
Nonanal	358	358	103	102	102.5
Nonanone-2	362	362	.....	38	.....
Decanal	358	358	.....	.....	.....
Undecanone-2	362	362	.....	.....	.....
Dodecanal	358	358	.....	.....	.....
Pentadecanone-2	362	362	.....	.....	.....
Unidentified	375	.....	88	.....	.....
Unidentified	390	.....	186	.....	.....
Unidentified	388–390	.....	168	.....	.....
Unidentified	377	.....	160–162	.....	.....

[a] Tentative identifications based on data from paper and column chromatograms.

The presence of methyl ketones in these products was not unexpected, since they have been reported previously (15) in dairy products undergoing substantial heat treatments. The precise origin of these compounds in milk fat, however, is not known at this time.

The results of this study indicate that fat oxidation had been initiated in the relatively good dry whole milk and had progressed to a greater extent in the deteriorated product. Saturated and unsaturated aldehydes are known products of lipid autoxidation. Tentative identification of nonanal as the only higher aldehyde in the good powder does not preclude the existence of other aldehydes. Failure to detect such compounds may be attributed to low concentrations and the lack of adequate methods of separation for various aldehyde-ketone DNP hydrazone mixtures as previously mentioned. Although data usually sufficient for conclusive identification were obtained on four unsaturated carbonyls in the case of the deteriorated product, the results could not be correlated with those for any known unsaturated carbonyls. Regeneration of the derivatives in question with one part H_2SO_4 and one part water resulted in odors suggesting the presence of unsaturated aldehydes.

The flavor characteristics of the two dry milks used in this investigation suggest that the differences lie in the type and concentration of carbonyls present. The deteriorated product had by far the largest concentration and variety of carbonyls. Whereas the relatively good dry whole milk was characterized mainly by the presence of methyl ketones, the deteriorated product contained both methyl ketones and saturated and unsaturated aldehydes. This suggests that differences in flavor of the products can be attributed to the aldehyde content of the powders. However, the term relatively good, used to describe the flavor characteristic of the better powder, indicates that it had a typical dry whole milk flavor. In this connection, it must be remembered that conventional dry whole milk at its best is not generally acceptable for beverage purposes from a flavor standpoint. Thus, the possibility that methyl ketones may contribute to the normal flavor of dry whole milk needs to be considered.

PART II. RELATION OF CARBONYL CONCENTRATIONS TO FLAVOR QUALITY

Part I of this report has shown that volatile carbonyl compounds are involved in flavor deterioration of stored dry whole milk. The second part of the investigation was undertaken to determine the type and relative concentrations of carbonyls in dry whole milks manufactured and stored under various conditions. Flavor studies on the reconstituted products before and after vacuum distillation and flavor evaluations of the distillates from these products were also conducted to establish whether any correlation exists between the off-flavors of the products and the type and quantities of volatile carbonyls they contain.

EXPERIMENTAL PROCEDURE

Samples: The quantitative determinations of this investigation were conducted on four samples of dry whole milk packed and stored under the following conditions:

Sample A—Packed in an atmosphere of nitrogen and stored at 40° F. for a period of 6 mo.

Sample B—Packed in an atmosphere of nitrogen and held at 40° F. for 27 mo.

Sample C—Vacuum-packed and held 3 mo. at 100° F., followed by further storage at 40° F. for 3 yr.

Sample D—Prepared with deodorized milk fat and stored for 9 mo. at 70° F. in an atmosphere of nitrogen.

A sample of pasteurized, homogenized milk was included as the control sample in this study. Ten pounds of Samples A, B, and C; 5 lb. of Sample D, and 4.85 gal. (5.0 lb. of milk solids) of the fresh product were employed.

Collection of volatile carbonyls and preparation of 2,4-DNP hydrazones. The collection of volatile carbonyls and their conversion to 2,4-DNP hydrazones were accomplished according to procedures reported in Part I. The DNP hydrazones were separated initially by the chromatographic methods A or B of Day *et al.* (4), employing 40-50 g. of celite. The forerun to the first band eluted from the columns was rechromatogrammed by Method A or the method of Corbin *et al.* (3), to isolate DNP hydrazones not observed on the column in the initial separation.

Each individual fraction separated by the column chromatographic procedures was divided into two equal parts. Tentative identification of the DNP hydrazones by ultraviolet studies and paper chromatography, using the methods of Gaddis and Ellis (6), Klein and de Jong (11), and Huelin (8), was performed on one part of the original fraction. The remaining part was used for quantitative studies.

Quantitative studies. For quantitative evaluation, the fractions were evaporated to dryness and redissolved in a definite volume of purified hexane or 95% ethanol. An accurately measured sample was streaked on filter paper and chromatogrammed either by the method of Gaddis and Ellis or that of Huelin. The separated bands were cut from the paper, extracted with 95% ethanol, filtered, and evaporated to dryness under vacuum. The DNP hydrazone residue was taken up in a definite volume of 95% ethanol and the optical density obtained at the wavelength of maximum absorption (previously determined by the qualitative studies). In general, the optical densities of the DNP hydrazones of aldehydes were made at 358 mμ, of ketones at 363 mμ, and of monounsaturated aldehydes at 375 mμ. Formaldehyde and acetaldehyde DNP hydrazones were analyzed at 345 and 356 mμ, respectively. Calculations for determining the concentrations of carbonyls in the various products were made as described by Wong *et al.* (15).

Flavor studies. Prior to distillation of the products, 10 g. of each was reconstituted with 80 ml. of distilled water. These samples, the distilled milks adjusted to normal total solids and the distillates, were evaluated by a taste panel of three experienced observers. Table 3 presents the flavor observations on these samples, together with the types of carbonyls found in the dry milks.

TABLE 3

Flavor and odor qualities of reconstituted dry whole milks before and after distillation as related to the types of carbonyl compounds detected in the distillates

Sample	Storage conditions	Flavor of product prior to vacuum distillation	Odor characteristics of distillate	Flavor of product after vacuum distillation	Type of carbonyls recovered
A	Nitrogen-packed Held 6 mo. at 40° F.	Rough, lactone, cooked	Cooked, cheesy, buttery, waxy, reminiscent of staleness	Rough, lactone, flat	Ketones
B	Nitrogen-packed Held 27 mo. at 40° F.	Typically stale, slightly oxidized	Old milk, aldehyde-like, oxidized, pungent	Flat, reasonably good product	Ketones, aldehydes, monounsaturated aldehydes
C	Vacuum-packed Held 3 mo. at 100° F. + 3 yr. at 40° F.	Stale, coconut, oxidized	Old milk, old fat, ketone-like	Flavor improved somewhat	Ketones
D	Nitrogen-packed, deodorized milk fat Held 9 mo. at 70° F.	Oxidized, stale	Old fat	Mild, musty, waxy, flat	Low molecular weight ketones aldehydes, unsaturated aldehydes
Pasteurized, homogenized fresh milk	.....	Normal milk flavor	Reminiscent of aldehydes	Flat	Low molecular weight ketones and aldehydes

RESULTS AND DISCUSSION

Concentrations of carbonyl compounds recorded in Table 4 are not absolute, quantitative data. Vacuum distillation under the conditions of this study does not allow for quantitative recoveries of volatiles from reconstituted dry whole milk. Moreover, it has been reported that the yields of carbonyls with DNP hydrazine differ between carbonyls (2, 15). However, details of the vacuum distillation and reaction period between carbonyls and reagent were standardized to eliminate any differences in the yields other than the actual differences in the products themselves. Thus, the concentrations listed in Table 4 are considered valid for the purpose of determining differences between the samples analyzed.

An analysis of the data presented in Table 3 indicates that with the possible exception of Sample C, vacuum distillation was effective in removing sufficient quantities of flavor-producing compounds, especially those related to staleness, to materially improve the flavor of the reconstituted products. Thus, the role of volatile carbonyls in the off-flavors of dry whole milk, as established in Part I of this study, is indicated again.

Data in Table 4 reveal that with the exception of the lower molecular weight aldehydes and ketones, the carbonyls identified are present as a result of heat

TABLE 4

Concentrations of carbonyls obtained from various dry whole milk products (mg. per 10 lb.)

Carbonyl	Sample				
	A	B	C	D[a]	Control
Acetone	2.01	5.63	2.34	1.66	24.71
Butanone-2	0.07	0.24	0.31	0.35	3.04
Pentanone-2	0.06	0.35	0.61	.....	0.39
Hexanone-2	0.30	1.20	0.97	0.17	0.23
Heptanone-2	0.27	0.44	0.96	0.02	.....
Nonanone-2	0.04	0.09	0.18	.....	.....
Undecanone-2	0.02	0.03	0.06	.....	.....
Tridecanone-2	0.01	0.01	0.02	.....	.....
Pentadecanone-2	0.01	.....	0.01	.....	.....
Formaldehyde	0.15	0.33	0.28	0.25	0.08
Acetaldehyde	0.22	0.84	1.05	0.22	0.08
Propanal	.....	0.14	.....	0.09	0.34
Pentanal	.....	0.54	.....	.....	.....
Hexanal	0.01	2.26	.....	0.54	.....
Heptanal	.....	0.94	.....	0.28	.....
Octanal	.....	0.35	.....	0.09	.....
Nonanal	.....	0.25	.....	0.02	.....
Decanal	.....	0.02	.....	0.16	.....
Dodecanal	.....	.....	.....	.....	.....
2-Pentenal	.....	0.02	.....	.....	.....
2-Hexenal	.....	0.03	.....	.....	.....
2-Heptenal	.....	0.29	.....	.....	.....
2-Octenal	.....	0.02	.....	.....	.....
2-Nonenal	.....	0.12	.....	.....	.....
2-Decenal	.....	0.06	.....	.....	.....
2-Undecenal	.....	0.03	.....	.....	.....

[a] Sample contained two mono- and two diunsaturated carbonyls of unknown identities.

processing and storage of the dry whole milks. The exact role of the different carbonyl classes in the various off-flavors is not clear. The dominant carbonyls in Samples B and D were saturated and unsaturated aldehydes, whereas Sample C contained only methyl ketones with the exception of formaldehyde and acetaldehyde, normal components of all products investigated. All three products were criticized as containing both oxidized and stale flavors. If the panel members were of one mind as to the nature of staleness, this suggests that the defect may be caused by more than one combination of compounds and that the off-flavor may change qualitatively with change in the concentrations of the responsible compounds. Although this study has not yielded entirely adequate data on the stale-oxidized relationship, the following scheme seems to account rather well for the information in Table 3.

	Carbonyl concentration		
	Low	Medium	High
Ketones		stale ⟶	oxidized
Aldehydes	stale ⟶	oxidized	

This scheme assumes that ketone formation somewhat precedes aldehyde formation, that in general aldehydes exhibit lower flavor thresholds than ketones, and that both classes of compounds can impart the impression of staleness near threshold. The need for threshold and flavor characterization studies on indi-

vidual as well as mixtures of carbonyls is quite evident before definite conclusions concerning their significance in staling can be drawn.

Deodorization of milk fat, prior to the manufacture of dry whole milk, is apparently an effective means of improving the final flavor of dry whole milk. Methyl ketones are noticeably absent in products prepared from deodorized milk fat. The formation and removal of odd-numbered methyl ketones during steam-stripping of butterfat has been reported (12). In addition to flavor improvement due to the removal of ketones, the deodorization process forms and removes lactones (13) which are responsible for the coconut-like flavor of dry whole milk (10). The identification of saturated aldehydes in the product prepared with deodorized milk fat indicates that although the product was originally nitrogen-packed, autoxidation apparently occurred in one or more of the five cans of sample used.

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A METHOD OF CONCENTRATING RIPENED CHEESE VOLATILES FOR GAS CHROMATOGRAPHY

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SUMMARY

A method has been developed for concentrating the volatile organic compounds in ripened cheese for gas chromatographic analysis. The method consists of a rapid, room temperature churning of a cheese slurry and centrifugation of the resulting heated butter. The dry aromatic oil which is obtained is subjected to high vacuum distillation and the distillates are fractionated. With fully mature Blue Cheese, an organic layer separates from the distillate and is easily isolated and dried. With Cheddar Cheese and immature Blue Cheese, the distillates are extracted with ether and the extract evaporated in the presence of a small amount of high-boiling solvent, benzyl alcohol, which acts as a carrier for the volatiles to be analyzed.

Gas chromatography is a helpful supplement to the analytical techniques commonly used in cheese ripening and flavor research. One difficulty in its use is that of obtaining a sample suitable for injection into the apparatus. A suitable sample, as defined here, is that obtained from the original product by a relatively nondestructive process which will neither alter the volatile compounds already present nor create new ones. This volatile organic fraction should be free from excess water and organic solvents which mask the chromatogram and should contain enough volatiles for several gas chromatographic separations.

Earlier, an atmospheric steam distillation of aqueous Cheddar and Blue Cheese slurries from 8 and 36 lb. of cheese, respectively, followed by ether extraction of the distillate was reported by Jackson *et al.* (4, 5). This method produces the best yield of volatile components but steam distillation, especially at atmospheric pressure, carries with it the danger of altering some of the existing volatile compounds and causing further hydrolysis of protein and fat. Patton (7) has shown that steam vacuum distillation of butter oil, a relatively mild treatment, can produce methyl ketones. Also, making an aqueous slurry from cheese usually involves the addition of an equal part of water. From 8 lb. of cheese, seven liters of slurry are produced. The distillation of this volume of material involves either a very large-scale distillation or a time-consuming series of smaller distillations.

The aroma of ripened cheese apparently predominates in the fat phase (4). With this in mind, an analytical technique was developed here which included among its steps a separation of oil from cheese, vacuum distillation of the oil, fractionation of the distillate, and gas chromatographic analysis of the volatile organic fraction. An explanation of this method is the essence of the present report.

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ANALYTICAL PROCEDURES AND RESULTS

Churning and separation of fat. One thousand grams of Blue or Cheddar Cheese is cut into small cubes and blendorized with an equal weight of water. The slurry, at approximately 20° C., is agitated by hand or mechanical stirrer and enough 2 *N* HCl is added slowly to lower the pH to 4.2. Soon butter granules rise to the top. After agitation is terminated the granules clump together. This churned slurry is cooled at 5° C. for 30 min.

The resulting butter is heated rapidly in a water bath to 48° C. and the melted oil is centrifuged in an International Size 2 centrifuge at 2,000 r.p.m. for 5 min. Three layers, oil, water, and protein, separate in the cups. The aqueous layer is removed with a pipette and the oil, containing small amounts of protein and moisture, is poured from the solid protein residue into new centrifuge cups containing 15 to 30 g. of anhydrous magnesium sulfate. After a 5-min. centrifugation, a clear, dry oil with cheese aroma results and is poured into a distillation flask.

Distillation. The distillation appartus consists of a one-liter round-bottomed flask connected directly to a vacuum trap which is connected to a T-shaped tube, one leg of which is attached to a McLeod gauge, the other to the vacuum pump (Figure 1). All connections and fittings are ground glass. After the vacuum pump is started, the contents of the distillation flask, agitated by a magnetic stirrer, are heated by an electrical heating mantle to 80° C. The vacuum trap is submersed in liquid nitrogen. When the pressure of the system is down to less than 5 μ of mercury, the stopcock leading to the vacuum pump is closed and the distillation of the closed system proceeds for 4–7 hr. In this time, the pressure increases to 25–50 μ (mercury). The vacuum is then released and the contents of the trap collected. For a test compound, ethyl butyrate (B.P. 121° C.), the yield of the distillation is approximately 30%.

Treatment of distillates and gas chromatographic analysis. Blue Cheese. The distillates obtained by the above procedure from fully ripened commercial Blue Cheese have a total volume of approximately 0.2–0.7 ml. and have separated into

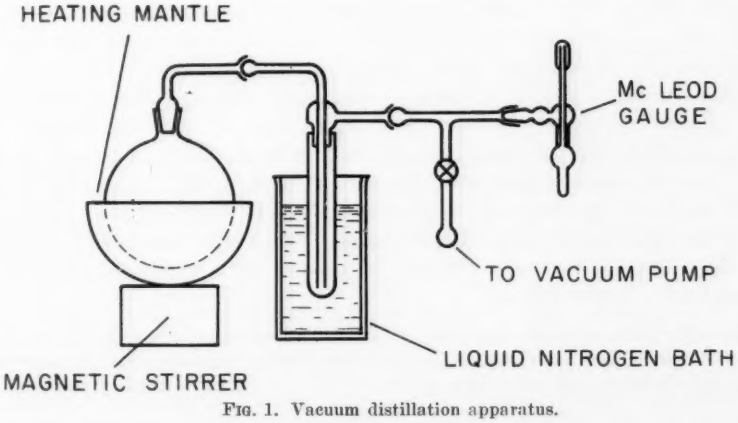

FIG. 1. Vacuum distillation apparatus.

two liquid layers. The aqueous phase (bottom) is removed with a syringe and a small amount of anhydrous magnesium sulfate is added to the remaining upper layer (organic). After standing overnight, the liquid is removed with a syringe from the settled drying agent. In this manner, a sample of pure organic volatiles (0.1–0.2 ml.) with an intense Blue Cheese aroma is obtained. A gas chromatogram of 18 μl. of a typical sample at the given conditions is presented (Figure 2). Peaks, shown here, represent only a portion of the total number of compounds. Under these conditions, 2-nonanone and other higher boiling components have retention times longer than 1 hr.

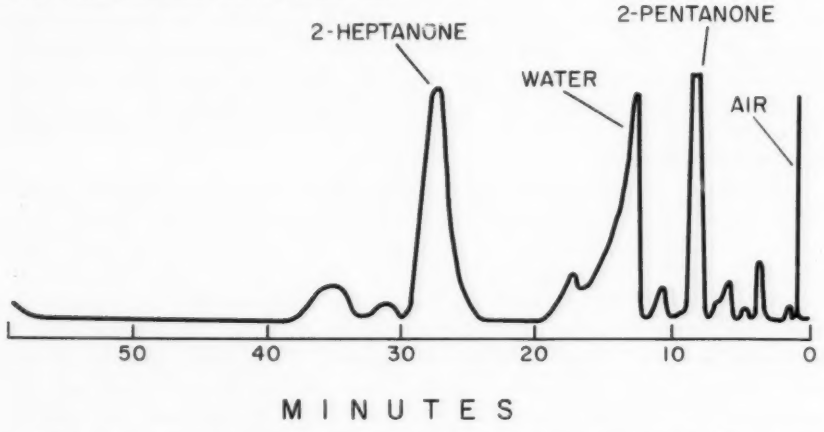

FIG. 2. Gas chromatogram of Blue Cheese volatiles.[2] Column temperature 80° C., 2 m.v. recorder span, flow rate 66 ml. per minute, column Carbowax 1540, filament current 270 m.a.

Distillates from underdeveloped or immature Blue Cheese fail to separate into layers and further fractionation by a technique described in the following section on Cheddar Cheese is necessary. Immature Blue Cheeses have a relatively hard body, a small amount of blue veins, and little flavor.

Cheddar Cheese. The distillates from fully ripened Cheddar Cheese (0.1–0.5 ml.) and those of underdeveloped Blue Cheese do not separate into layers even when saturated with potassium carbonate. To eliminate the excess water, which obscures the presence of certain compounds, the distillate is extracted with 3 ml. of ether in 1-ml. portions and the extract is dried with anhydrous magnesium sulfate. To the dried extract is added 50 μl. of benzyl alcohol (B.P. 205° C.), and the ether is evaporated from the mixture at 35° C. until the ether aroma is no longer detectable in the liquid residue. If the benzyl alcohol is not added to the ether extract, a large percentage of the volatile compounds will be lost in the evaporation to near dryness, because of their relatively low boiling points and trace concentrations.

[2] Compounds indicated in this and other figures have been identified tentatively by retention time and functional group test. Only acetic and butyric acid in Figure 4 have been identified positively by infrared spectrophotometry.

The benzyl alcohol retains the desired compounds and is a suitable carrier for injection into the gas chromatography apparatus. It does not interfere with the chromatogram because of its high boiling point and it will remain in the column until the temperature is raised far above the temperature of analysis. Gas chromatographic examination of 25 μl. of pure benzyl alcohol shows no impurities.

A typical gas chromatogram of the lower boiling point components in a 25-μl. sample obtained by the above technique from the distillate of an aged Cheddar Cheese is shown (Figure 3).

For the analysis of some compounds in the aged Cheddar Cheese distillate the final ether extraction step is not necessary. If 100 μl. of the distillate is directly injected into the gas chromatography apparatus fitted with a Carbowax 1540 column at 60° C. and a helium flow rate of 162 ml/min, a satisfactory chromatogram of polar compounds with boiling points of less than 100° C. (approx.) and nonpolar compounds with boiling points less than 130 ° C. is possible (Figure 4). The peaks of these compounds will appear before the water

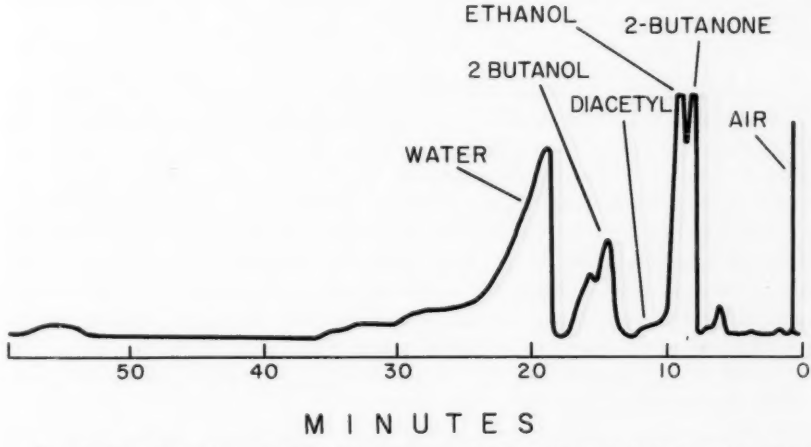

FIG. 3. Gas chromatogram of Cheddar Cheese volatiles in benzyl alcohol carrier. Column temperature 67° C., 2 m.v. recorder span, flow rate 50 ml. per minute, column Carbowax 1540, filament current 278 m.a.

peak, which is delayed on the polar column. Also, by raising the temperature of the column to 150° C. upon appearance of the water peak, the water will be quickly eluted and the high boiling compounds will follow. Decreasing the recorder sensitivity to 10 mv. minimizes the base line change, due to the temperature increase.[3]

[3] The gas chromatography apparatus used in this research was an Aerograph Model A-100 equipped with a Varian recorder with a 1–10 mv. range selector switch. With other commercial apparatuses having thermal conductivity detectors, the described technique may result in a much greater base line change.

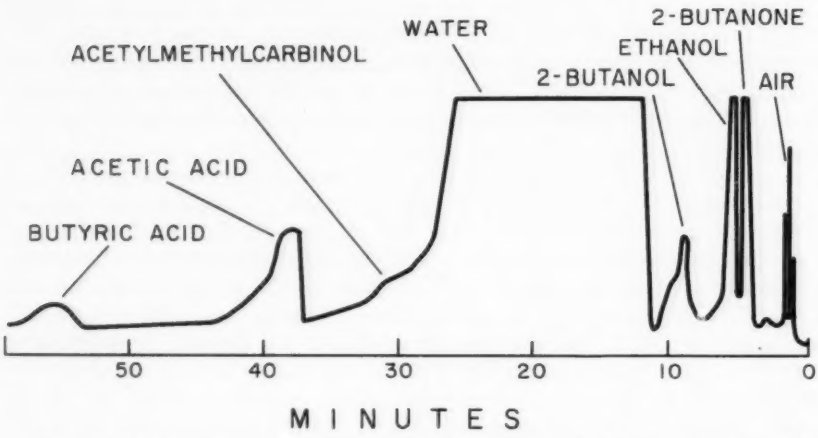

FIG. 4. Gas chromatogram of distillate from Cheddar Cheese oil. Column temperature 60° C. until water peak, recorder span 2 m.v. before water peak, 10 m.v. after water peak, flow rate 162 ml. per minute, column Carbowax 1540, filament current 273 m.a.

DISCUSSION

By removing the oil from cheese, a quick separation of a good portion of the volatiles from the easily decomposed protein and the bulk of the water is accomplished. Previous workers have separated the oil from cheese by a variety of methods which include centrifuging cheese slurries at 90° C. (1), pressing cheese-sand mixtures in a hydraulic press (6), and churning cheese-water mixtures by mechanical agitation at 40° C. for 3 hr., followed by ether extraction (3). The present churning technique has the advantages of being very rapid and accomplished at below room temperatures. Heat treatment is kept to a minimum in the separation of oil from the resulting butter which oils off easily. The yield of oil, 60%, is far greater than the yields obtained by using a hydraulic press or centrifuging cheese slurries in this laboratory.

The churning rate depends upon pH and temperature. Agitation of the slurry at pH 4.0–4.2 and approximately 20° C. results in almost instantaneous churning. Lower temperatures and higher pH's prolong the process considerably. This optimum pH range is the same as that in which the churning of cream occurs most rapidly (2).

The pH adjustment serves another purpose. At the normal pH of ripened Blue and Cheddar Cheese, approximately 6.0 and 5.4, respectively, the fatty acids are predominantly in the salt form, whereas at pH 4.2 they are predominantly in the acid form and can partition between the water and fat phase.

High-vacuum distillation is a fairly efficient method of stripping the desired volatiles from the tenacious oil at moderate temperatures. Since only the cheese oil is distilled, it is possible to keep the distillation on a small scale, even when starting with several pounds of cheese.

At no time in the entire procedure are quantities of solvents employed which might cause artifacts due to the presence of impurities.

The yields of volatile components, by the described techniques, vary with the variety of cheese used and the degree of ripening of the cheese. Fully ripened Blue Cheese gives enough pure volatile essence from 2.2 lb. of cheese for detailed analysis on several types of column under varying conditions. Ripened Cheddar gives appreciably less, but still enough for two or three separations.

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ENZYMIC DEGRADATION OF β-CASEIN BY A SNAKE VENOM PREPARATION

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SUMMARY

The action of a snake venom preparation from *Crotalus adamanteus* on β-casein has been studied. The action resulted in the formation of a turbidity, which was measured with time in the spectrophotometer. The effects of temperature, metal ions, pH, and ionic nature of the medium have been observed. The reaction appears to be proteolytic in nature, being accompanied by an increase in ninhydrin-positive material and resulting in the formation of at least four different fractions from β-casein. The proteolytic activity always preceded the precipitate formation. The material precipitated during the reaction was essentially phosphorus-free and comprised 20-30% of the original β-casein.

During the course of a study on the nature of the phosphate linkages in casein fractions, it had been noted that when β-casein is incubated with a snake venom preparation having phosphodiesterase activity, turbidities developed (9). This phenomenon is accompanied by a shift in pH toward the acid side, as had been previously noted by Perlmann (14), who took this to mean the cleavage of phosphodiester bonds. However, this conclusion has been recently challenged (7, 9). The present communication reports some of the properties and characteristics of the reaction of β-casein with an enzyme preparation obtained from *Crotalus adamanteus*.

MATERIALS AND METHODS

β-Casein. The casein was prepared from whole casein by fractionation in aqueous urea solutions (6). The preparation used in these studies had a moisture content of 7.62% and a total phosphorus content of 0.61% on a moisture-free basis.

Enzyme preparation. The preparation used was obtained as a dry powder from Ross Allen's Reptile Institute, Silver Springs, Florida, and was fractionated by the method of Sinsheimer and Koerner (16). The precipitate formed with 0-40% acetone was found to be most active in causing turbidity with β-casein. This fraction, obtained in the first acetone cycle, was dissolved in water and used as the enzyme preparation. It also had phosphodiesterase activity. It contained 8 mg. protein/milliliter as determined by the micro-Kjeldahl procedure, using the factor 6.25. The enzyme solution retains full activity when stored at 4° C. for 3 mo.

Measurement of turbidity. The turbidity measurements were made in the Beckman Spectrophotometer (Model DU) at a wave length of 600 mμ. The reaction was carried out in cuvettes of 1-cm. path length at a constant temperature maintained by means of thermospacers.

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Measurement of proteolysis and pH. The proteolysis was measured by the increase of ninhydrin-positive material of the entire reaction mixture as described previously (9). The modified ninhydrin method of Moore and Stein (12) was employed, and the results were quantitated by comparison with a standard leucine curve. It is necessary to keep in mind that the quantitative data are taken only as an approximation of the degree of proteolysis, for reasons previously stated (9). The pH determinations were made at room temperature before and after the reaction, using the glass electrode.

Measurement of nitrogen and phosphorus. Total nitrogen was determined by a modification of the micro-Kjeldahl method of Ma and Zuazaga (10), in which a mixture of K_2SO_4 and HgO was used as catalyst during the digestion procedure. Inorganic orthophosphate was determined on trichloroacetic acid filtrates (final concentration—10% TCA) of protein solutions by the method of Fiske and Subbarow (5). Total phosphorus was determined as above after prior digestion with H_2SO_4 (17).

EXPERIMENTAL PROCEDURE

The following types of experiments were carried out to study the properties and characteristics of the action on β-casein of the snake venom fraction noted above.

Turbidimetric studies. In these studies, the desired amount of enzyme was placed in the cuvette, which was then allowed to come to temperature, usually 38° C., unless otherwise indicated, in the spectrophotometer. The remaining solution, including the casein and any other substances pertinent to the experiment, was placed in a tube immersed in a water bath at 38° C. At zero time, the contents of the tube was added to the cuvette to bring the final volume to 3.0 ml. The pH of the solution in the tube had been previously adjusted, and since the volume of the enzyme solution was, in general, less than 0.2 ml., there was very little change in pH. This was verified by independent mixing experiments. The first reading of the optical density was made 1 min. after mixing, and readings were continued at intervals until 30 min. had elapsed, as indicated in the accompanying figures. The pH was then measured, and a plot of the increase of optical density at 600 mμ with time was made. The exact composition of the reaction media is given below, under Results.

Proteolytic studies. These studies were carried out separately from the turbidimetric studies. Tubes were incubated in a water bath at 37° C.; 0.1-ml. aliquots of the reaction mixture were taken at zero time and every 3 min. thereafter until the fifteenth minute, when aliquots were taken every 5 min. until the experiment was concluded after 30 min. The increase of ninhydrin positive material was measured as previously described (9) and compared with the turbidimetric data. Data are reported in Table 1 in terms of bonds split/30,000 mol. wt.

Distribution of nitrogen and phosphorus. To determine the distribution of N and P between the supernatant and precipitate, the following experiment was performed. Two reaction tubes containing 75 mg. of β-casein, 0.75 mg. of

TABLE 1

Extent of proteolysis during the action of snake venom on β-casein

System	Bonds hydrolyzed [a] (30,000 mol. wt.) After 6 min.	At end 30 min.
Control [b]	1.2	3.7
Control (T = 26° C.)	1.0	3.2
$-Mg^{++}$	1.3	2.4
$\frac{\text{Substrate}}{\text{Enzyme}} = \frac{400}{1}$	0.7	2.1
$+ 3.3 \times 10^{-3}$ *M* versene ($-Mg^{++}$)	0	0
$+ 3.3 \times 10^{-2}$ *M* versene ($-Mg^{++}$)	0	0
$+ 8.2 \times 10^{-3}$ *M* veronal	0.8	2.5
$+ 3.3 \times 10^{-1}$ *M* veronal	0	0
$+ 3.3 \times 10^{-1}$ *M* NaCl	0.7	2.7
$+ 6.6 \times 10^{-1}$ *M* NaCl	1.2	2.7

[a] All values reported are the averages of at least two independent determinations.

[b] Control system consisted of a substrate-to-enzyme ratio of $\frac{100}{1}$ in an unbuffered medium of pH 9.5 at 36° C. containing 3.3×10^{-3} *M* $MgCl_2$. All other systems were the same except as indicated.

enzyme, and 50 μmoles of Mg^{++} in a volume of 10 ml., pH 9.7, were incubated at 36° C. A third control tube contained no enzyme. Aliquots were taken at 0, 15, and 30 min. to determine total N, total P, and inorganic P of the supernatant after the solution had been clarified by centrifugation at room temperature at 2,000 g for 30 min. The extent of proteolysis was also measured, using the whole reaction mixture as described above, and the pH was also determined at the end of 30 min. The precipitate could be further fractionated, as will be described under Results and Discussion.

RESULTS

Turbidimetric studies. Figure 1 is the plot of a typical experiment in which snake venom was incubated at 38° C. in a final volume of 3.0 ml. with β-casein (0.5%) (substrate to enzyme weight ratio of 100:1). The reaction underwent a pH shift of 1.2 units toward the acid side from an initial pH of 9.5, after correcting for pH changes in the control. Also present in the medium was $MgCl_2$ (3.3×10^{-3} *M*). It is noted that an S-shaped curve was obtained with a lag of about 4 min. and with a slope of $\frac{0.243 \text{ O.D. units}}{\text{minutes}}$ for the straight line portion of the curve (between 4 and 11 min.). When the enzyme preparation was boiled for 5 min. and then incubated with the casein under the same conditions, there was no reaction. The addition of boiled enzyme to the system described above was neither inhibitory nor stimulatory. When the substrate-to-enzyme ratio was varied over an eightfold range by varying the enzyme concentration, the series of plots illustrated in Figure 2 was obtained. As the β-casein to snake venom ratio increased, the lag period increased, and the slope of the straight-line portion of the curve decreased, as did the pH shift. Also shown in Figure 2 is the plot obtained when the temperature of the reaction was reduced to 25° C. with a substrate-to-enzyme ratio of 100:1. The lag

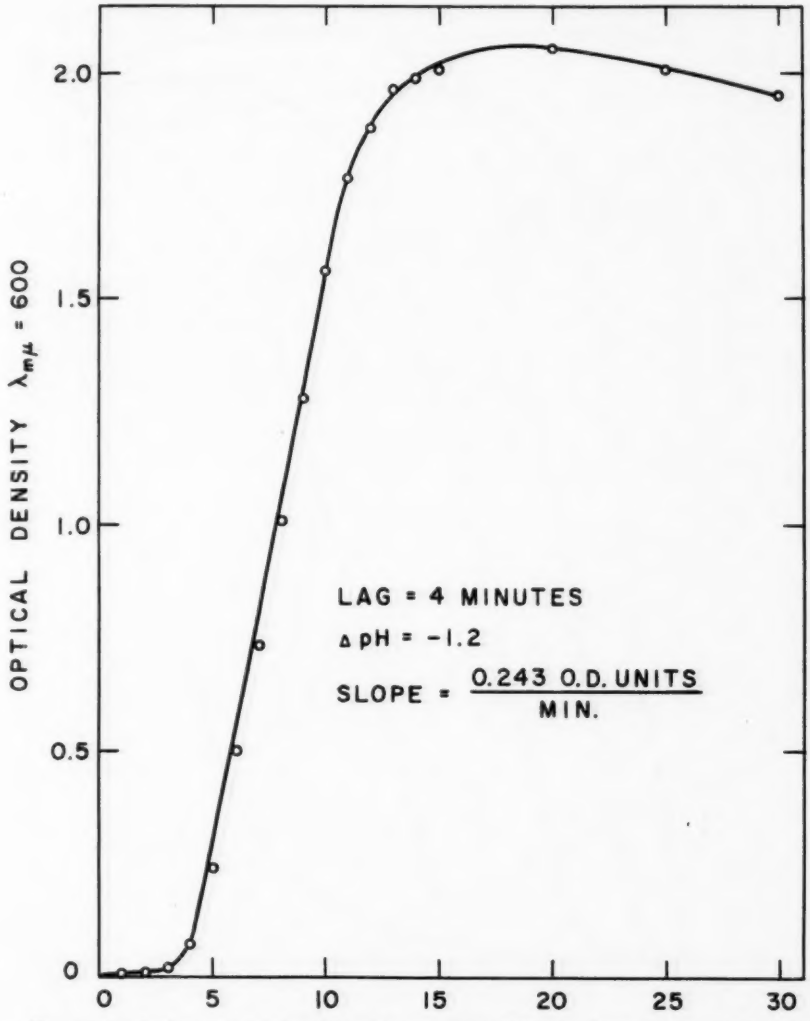

FIG. 1. Incubation of β-casein with snake venom at 38° C. in the presence of 3.3×10^{-3} *M* $MgCl_2$ at an initial pH of 9.5.

period was greatly extended, and the slope was reduced by approximately one-half.

It was found that when calcium ions replaced magnesium, the reaction proceeded at the same rate as in the presence of magnesium. However, the omission of divalent metal ions resulted in an increase in the lag and decrease in the slope, as seen in Figure 3. The addition of boiled enzyme to the system

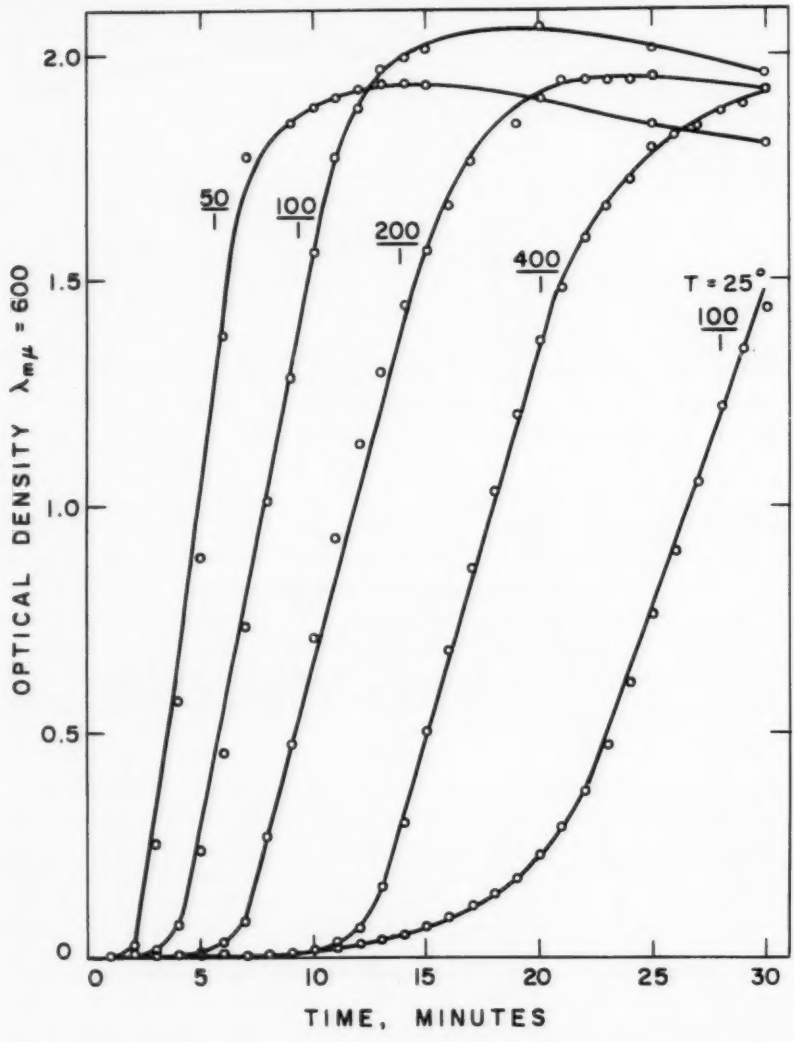

FIG. 2. Incubation of β-casein with snake venom at 38° C.—varying substrate to enzyme concentration in the presence of 3.3×10^{-3} M $MgCl_2$ at an initial pH of 9.5. $(\frac{50}{1})$ slope = $\frac{0.348 \text{ O.D. units}}{\text{minutes}}$, $\triangle pH = -1.4$, $(\frac{100}{1})$ slope = $\frac{0.243 \text{ O.D. units}}{\text{minutes}}$, $\triangle pH = -1.2$, $(\frac{200}{1})$ slope = $\frac{0.185 \text{ O.D. units}}{\text{minutes}}$, $\triangle pH = -1.0$, $(\frac{400}{1})$ slope = $\frac{0.166 \text{ O.D. units}}{\text{minutes}}$, $\triangle pH = -0.6$. T = 25° C. $(\frac{100}{1})$ slope = $\frac{0.133 \text{ O.D. units}}{\text{minutes}}$, $\triangle pH = -1.1$.

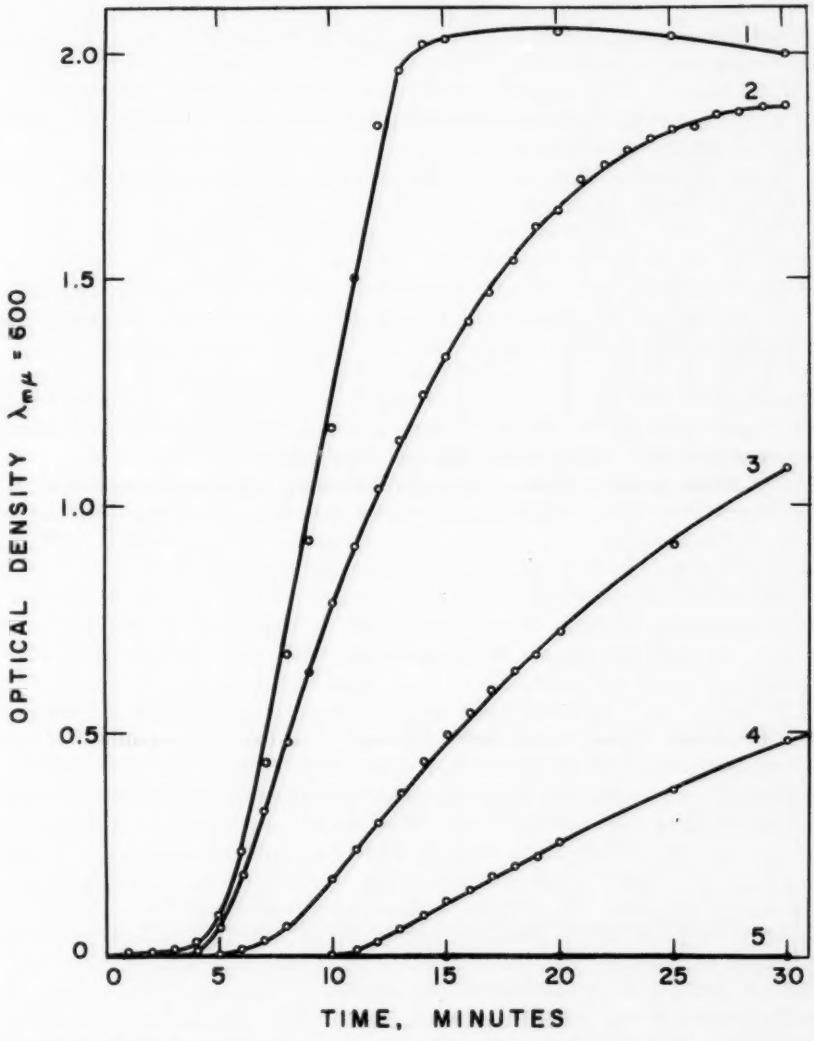

FIG. 3. Effect of magnesium and versene on the reaction of β-casein and snake venom at 38° C. and initial pH 9.5. Substrate-to-enzyme ratio $\frac{100}{1}$ (1) + 3.3×10^{-3} *M* $MgCl_2$ slope = $\frac{0.249 \text{ O.D. units}}{\text{minutes}}$, ΔpH = −1.1 (2) 3.3×10^{-3} *M* $MgCl_2$ + 3.3×10^{-3} *M* versene slope = $\frac{0.139 \text{ O.D. units}}{\text{minutes}}$, ΔpH = −1.2 (3) $-MgCl_2$ slope = $\frac{0.058 \text{ O.D. units}}{\text{minutes}}$, ΔpH = −0.40 (4) $-MgCl_2$ + 3.3×10^{-3} *M* versene slope = $\frac{0.025 \text{ O.D. units}}{\text{minutes}}$, ΔpH = −0.10 (5) $-MgCl_2$ +3.3×10^{-3} *M* versene slope = 0 ΔpH = 0.

containing no magnesium is without effect. Versene, on the other hand, inhibited the reaction, and this inhibition could be overcome by the addition of magnesium ions, as is also seen in Figure 3. It was also found in this connection that the β-casein had no major metal contamination, so that the activity in the absence of added divalent metals must be due to trace contaminants in the casein or enzyme preparation.

When the initial pH of the reaction was varied between 6.2 and 9.9 in the unbuffered system, there was no change in the lag period (4-6 min.), but a decreasing shift in pH was observed with decreasing initial pH until pH 7.5, where no pH shift was observed. The value for the slope of the straight line portion of the curve did not vary appreciably over the pH range investigated. When the reaction was carried out in varying concentrations of veronal buffer at pH 9.5, it was noted that the reaction was inhibited, as can be seen in Figure 4. Increasing concentration of NaCl also inhibited the system, but for reasons different from veronal, as will be shown below. It is also seen in Figure 4 that somewhat higher NaCl concentrations were required to achieve inhibitions comparable to those with veronal (see legend of Figure 4).

Proteolytic studies. It is well known that snake venoms possess many enzymic activities (18), including proteolytic activities (4). The preparation from *C. adamanteus* was treated with diisopropylfluorophosphate (DFP), which is known to inhibit trypsin and chymotrypsin (4), in a manner previously described (9), and allowed to react with β-casein. The casein was degraded as before without inhibition. When the reaction was followed by measuring the increase in ninhydrin-positive material, it was found that after 30 min., there were about four bonds hydrolyzed, even when the enzyme was treated with DFP. Table 1 gives the results of a series of experiments in which the reaction of snake venom with β-casein was followed by the ninhydrin method. It can be seen that about four bonds are hydrolyzed after 30 min., but hydrolysis can be detected very early, even before turbidity formation can be visually detected. It would appear that the effect of a reduction in temperature is primarily to increase the lag period, since proteolysis was not influenced greatly. In this connection Berridge (2) has shown that the temperature coefficient for aggregation of para-casein with calcium ions is much greater than the temperature coefficient for the rennin-catalyzed proteolysis of casein. This finding was confirmed by Claesson and Nitschmann (3) and extended to other casein systems by Zittle and Pepper (20). The absence of magnesium ions from the medium reduced the over-all hydrolysis, and when versene was added, the proteolytic activity was abolished. This was true even with 3.3×10^{-3} *M* versene, which did not prevent the appearance of a slight turbidity [see Figure 3, Plot (4)]. It is interesting to note that high concentrations of veronal buffer inhibit proteolysis as well as turbidity formation, whereas the highest NaCl concentrations used inhibited proteolysis by about 25%. These salt concentrations did inhibit the turbidity development, especially at 6.6×10^{-1} *M* NaCl [See Figure 4, Plot (7, 8)]. It can be speculated that veronal, at high concentrations, inhibits the proteolytic enzyme, whereas NaCl merely inhibits

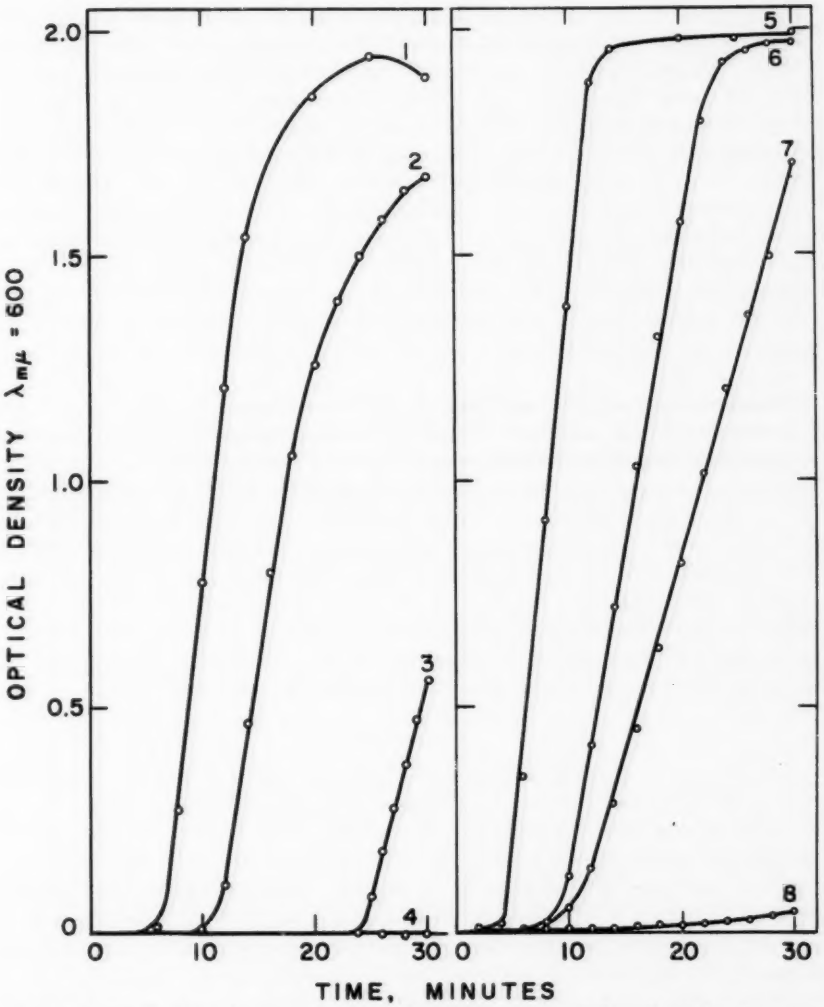

FIG. 4. Effect of varying veronal and NaCl on the reaction of β-casein and snake venom at 38° C. and initial pH = 9.5. Substrate-to-enzyme ratio $\frac{100}{1}$. (1) 3.3×10^{-2} *M* veronal slope $= \frac{0.207 \text{ O.D. units}}{\text{minutes}}$, $\Delta pH = -0.2$ (2) 8.2×10^{-2} *M* veronal slope $= \frac{0.151 \text{ O.D. units}}{\text{minutes}}$, $\Delta pH = 0$ (3) 1.6×10^{-1} *M* veronal slope $= \frac{0.094 \text{ O.D. units}}{\text{minutes}}$, $\Delta pH = 0$ (4) 3.3×10^{-1} *M* veronal slope = 0 $\Delta pH = 0$. (5) 3.3×10^{-2} *M* NaCl slope $= \frac{0.251 \text{ O.D. units}}{\text{minutes}}$, $\Delta pH = -0.9$ (6) 1.6×10^{-1} *M* NaCl slope $= \frac{0.139 \text{ O.D. units}}{\text{minutes}}$, $\Delta pH = -0.60$ (7) 3.3×10^{-1} *M* NaCl slope $= \frac{0.088 \text{ O.D. units}}{\text{minutes}}$, $\Delta pH = -0.7$ (8) 6.6×10^{-1} *M* NaCl slope $\cong 0$ $\Delta pH = 0$.

the formation of the precipitate. This remains to be investigated further. As further evidence that a proteolytic reaction has taken place, the precipitate was collected by centrifugation, and crudely fractionated into at least three fractions. This was accomplished as follows: The precipitate was dissolved with 0.1 *N* NaOH and immediately dialyzed against water at 4° C. for 48 hr., with one change of the dialysis medium. This procedure resulted in a precipitate which was removed by centrifugation in the cold (ca. 10% of original N). The supernatant was allowed to stand at 37° C. under toluene, which resulted in the appearance of a second precipitate which was again separated by centrifugation, this time at room temperature (ca. 2.5% of original *N*). The third fraction was obtained by lyophilization of the supernatant solution (ca. 10-15% of original *N*). It was also found that patterns obtained by moving boundary electrophoresis of the original precipitate indicated a minimum of three fractions.

Distribution of nitrogen and phosphorus. Snake venom was allowed to react with β-casein for 30 min., and the precipitate was removed by centrifugation. It was then found that the supernatant contained 70-80% of the original nitrogen of the medium, whereas it contained essentially 100% of the phosphorus. No inorganic phosphorus was formed during the course of the reaction. All the phosphorus of the original β-casein fraction remained organically bound in the soluble fraction. It was also found that upon paper electrophoresis (veronal buffer pH 8.5 $\mu = 0.05$, 100 V, 15.5 hr.), a component of the supernatant migrated to the positive pole with a greater mobility than β-casein, indicating a greater number of negative charges. Under the some conditions, the precipitated material moved toward the negative electrode.

DISCUSSION

The action of various proteolytic enzymes on casein has been extensively studied by Mattenheimer and Nitschmann (11). These workers observed that where casein became clottable by calcium ions, the reaction velocity curves showed two phases, a primary reaction yielding about 2% nonprotein-nitrogen (NPN), very rapidly followed by a general proteolytic breakdown, giving straight curves up to much higher NPN values. The clotting point was always near the end point of the primary reaction. However, when α-casein and β-casein were compared, it was found that rennin (13) and pepsin (19) act on α-casein in the specific manner described above, whereas these enzymes hydrolyzed β-casein in a nonspecific manner (straight NPN/time curve). The present study would seem to indicate that the reaction of snake venom on β-casein is generally akin to the action of rennin and pepsin on α-casein. There is a rapid proteolysis before turbidity can be detected, which is then accompanied by a further increase in proteolysis. The Carlsberg group have advanced a rational two-step theory to explain the clotting of milk by rennin (8). This theory states that the clot is due to precipitation of calcium paracasein after rennin-catalyzed proteolysis of the original casein. In addition, the S-shaped curves obtained are similar to that obtained for the action of rennin on skimmilk (3).

The present reaction is similar in some respects to the action of trypsin on β-casein (15). In the latter case, on short-time incubations, a phosphorus-free fraction was obtained which consisted of 25% of the total nitrogen. Such a fraction was obtained in the present study, under somewhat different conditions, e.g., pH. In addition, the snake venom also showed a definite metal requirement and was inhibited by versene, whereas trypsin is not inhibited by this reagent (4).

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CHANGES IN pH OF MILK DURING FREEZING AND FROZEN STORAGE [1]

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SUMMARY

Measurement of pH during freezing and frozen storage of pasteurized and raw milk and of raw milk with added calcium, phosphate, and citrate showed that marked pH changes occurred in frozen milk. During slow freezing, the pH of milk decreased to values as low as 5.8, whereas little change in pH occurred during fast freezing. During storage at −7° C. as well as at −12° C. the pH decreased to a minimum during the first 2 to 3 wk. of storage, and increased gradually thereafter. The pH minimum of pasteurized milk and raw milk with added salt (5.8-6.0) was generally lower than that of raw milk (6.1-6.2). The increase in pH after the pH minimum was reached was more gradual in pasteurized milk and raw milk with added chemicals than in raw milk. Although the amount of precipitated protein was measured simultaneously with pH, the data did not indicate a definite relation between pH changes and protein precipitation.

Changes in the pH of millk during freezing and frozen storage have been suggested as a causative factor in protein destabilization in frozen milk (4, 5). Information on such pH changes, however, is limited, presumably because of the difficulties involved in measuring pH at temperatures below freezing. It would appear that the only pertinent work is that of Tessier and Rose (5) who showed that significant changes in pH do occur. Using the technique of centrifugation to separate ice and liquid, they measured pH values as low as 5.8 at −7° C. Recently developed electrodes which will allow pH measurements at below-freezing temperatures without separating liquid and ice phases (9) offered a new approach to the problem. This paper presents the results of a study of pH changes in milk during freezing and subsequent storage using these electrodes.

EXPERIMENTAL PROCEDURE

Milk obtained from morning milkings at the Central Experimental Farm, Ottawa, was separated, pasteurized (when required) at 65° C. for 30 min., cooled to about 2° C., and then frozen immediately in metal cans, using seeding with ice to reduce supercooling.

Samples in which the pH was measured during freezing were frozen by cooling in agitated brine from −1° C. initial temperature at rates of 1° C/hour or more (fast), 0.25° C/hour (moderately slow), and 0.1° C/hour or less (slow freezing). Several samples of the same milk were frozen simultaneously and samples were removed at each desired temperature and analyzed. Most freezing tests were made with raw milk, but pasteurized milk and milk ultrafiltrates also were used.

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Most storage tests were made at −12° C. (+10° F.), but a few were made at −7° C. (+20° F.). Four-hundred-milliliter samples in 1-pt. tin cans were frozen in less than 12 hr. (fast freezing) in air at the storage temperature. Raw milk, pasteurized milk, and raw milk to which potassium citrate (10 m*M*), potassium phosphate (5 m*M* mono-, 5 m*M* di-), or calcium chloride (10 m*M*) was added, was used in these tests. Representative portions for measurement of pH and amount of precipitated protein were removed at intervals by uniformly scraping over the surface of the samples; the first scrapings were always discarded because of possible surface desiccation.

The method of pH measurement has been discussed in detail previously (9). In brief, a saturated calomel electrode, modified by adding glycerol to the saturated KCl solution [final concentration 50% (wt. KCl-free basis)] to prevent solidification of the solution below −10.7° C., and a mercury-filled glass electrode with a pH-sensitive glass membrane area of about 5 cm.^{2}, were used and the potential measured with a Beckman Model G pH meter. The scraped and finely crushed frozen material was packed around the electrodes. The electrodes were standardized at the sample temperature in a solution containing 0.16 *M* potassium phosphate in a 50% glycerol–50% water mixture (wt/wt) and having a pH of 7.0 ± 0.05 between −30 and +40° C. (9). Measurements in different locations of a given sample were reproducible within ± 0.1 pH unit.

The amount of protein precipitated was determined by centrifuging 15 or 50 ml. of milk (thawed at 4° C. overnight) for 10 min. at about 700 × g at the tip (3). The amount of precipitate was expressed on a per cent by volume basis.

RESULTS

Marked decreases in pH to values as low as 5.8 generally occurred during slow freezing, whereas the effect of fast freezing on pH was small in all tests (Figure 1). During slow freezing the pH tended to increase again after the pH minimum was reached at about −10° C. Comparable results were obtained for raw milk, pasteurized milk, and milk ultrafiltrate. The pH of different samples frozen under the same conditions varied by up to 0.3 pH unit.

Changes in pH during storage were similar in most instances, regardless of treatment (Figures 2-4). The pH decreased markedly at the beginning of the storage period to values 0.5 to 1.0 unit below the prefreezing pH and increased gradually thereafter. No significant differences were noted between raw milk samples stored at −12 and at −7° C. (Figures 2 and 4). The variability in pH between different samples of the same milk and of different milks was relatively small in pasteurized milk and raw milk with added chemicals (within ± 0.15 pH unit), but larger in raw milk. This larger variability is mainly due to differences in the minimum pH value and the time of its occurrence.

There was no significant difference between the effects of pasteurization and added chemicals (Figures 2 and 3). In addition to improving the reproducibility, compared with raw milk, addition of chemicals and to a smaller

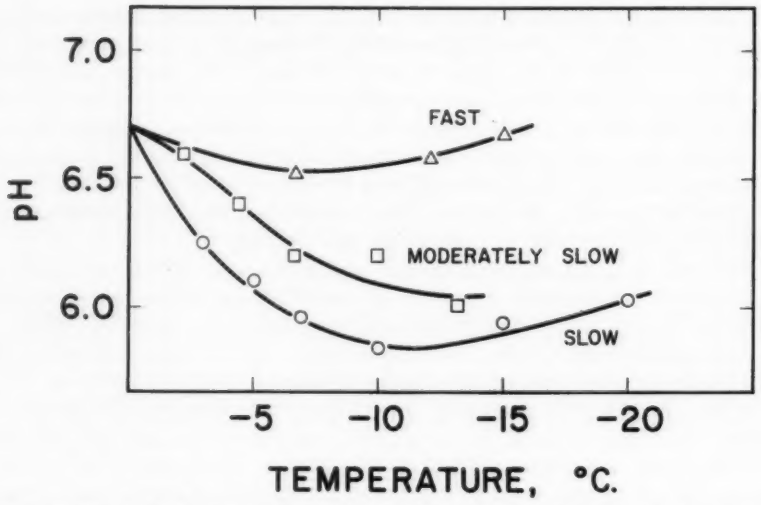

FIG. 1. Effect of freezing rate on the pH of raw milk during freezing (averages of two tests).

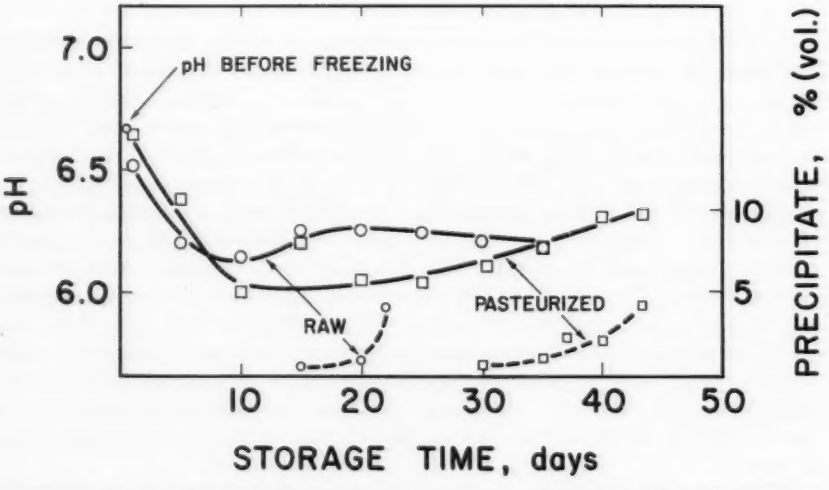

FIG. 2. Changes in pH (solid lines) and amount of protein precipitated (dotted lines) for samples of raw and pasteurized milk during storage at −12° C. (+10° F.) (average of five and two tests, respectively).

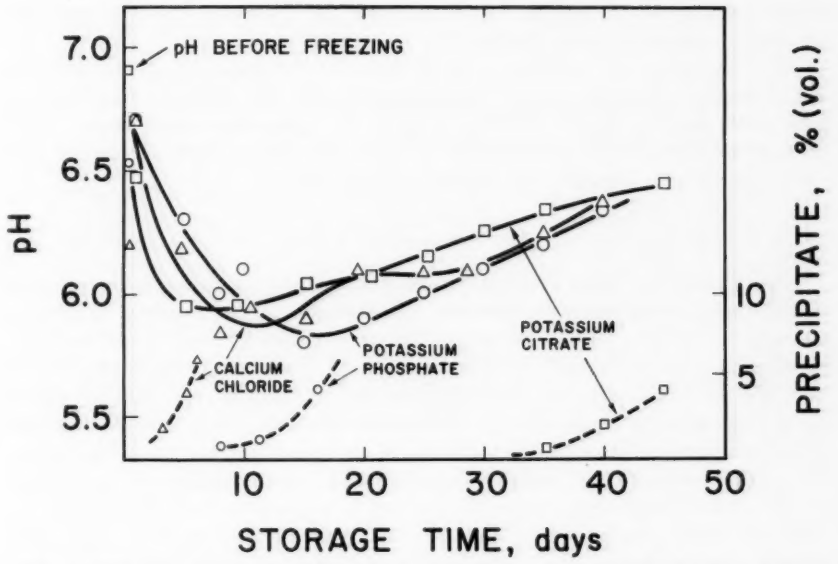

FIG. 3. Changes in pH (solid lines) and amount of protein precipitated (dotted lines) for samples of raw milk to which chemicals had been added [stored at −12° C. (+10° F.), averages of two tests].

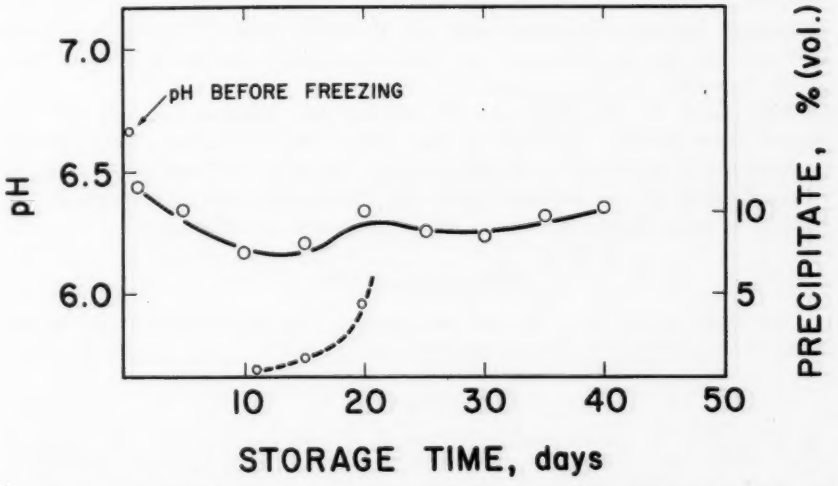

FIG. 4. Change in pH (solid line) and amount of protein precipitated (dotted line) for samples of raw milk during storage at −7° C. (+20° F.) (average of five tests).

extent pasteurization tended to decrease the pH minimum and to make the subsequent increase more gradual.

The beginning of protein precipitation did not always coincide with the pH minimum (Figures 2-4). Precipitation of protein started earlier in raw milk with added calcium chloride or potassium phosphate than in raw milk without additives. Both pasteurization and addition of citrate retarded protein precipitation significantly (Figures 2 and 3).

DISCUSSION

Precipitation of salts from the supersaturated liquid phase of frozen milk is considered to be the main cause of pH changes during freezing and frozen storage (5, 8, 10). The decrease in pH is presumably due to precipitation of calcium phosphate and to a lesser extent of disodium phosphate and probably sodium carbonate (5, 10); whereas, the increase in pH may be caused by precipitation of such salts as monopotassium phosphate, monosodium and potassium citrate. Dissolution of part of the salts precipitated during the initial pH decrease (e.g., calcium phosphate) also may contribute to an increase in pH. The storage temperature would not be expected to affect markedly the types of salts precipitating, because most of the precipitating salts have eutectic points above −7° C. The relative amounts of precipitated salts, however, were presumably affected by storage temperature, and also by addition of salts, but this was not apparent in the pH changes measured.

The slowness of pH changes in milk indicates the rate at which salts precipitated in frozen milk. These rates are similar to those reported for lactose crystallization in milk (6, 7) and ice formation in high viscosity systems (1, 2). Insufficient crystallization nuclei and slow diffusion of the supersaturated compounds through the frozen mass are the main factors responsible for the slow and varying precipitation rates and presumably also cause a large variability in results between samples.

The results of this study do not indicate any relation between pH and protein precipitation. This may be due to the complex nature of the changes in composition associated with pH changes. Further information on changes in composition of the unfrozen phase in frozen milk during storage will be needed to understand the factors affecting protein precipitation.

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THERMAL INACTIVATION STUDIES ON PATHOGENIC BACTERIA IN MILK AND VARIOUS MILK PRODUCTS. I. *CORYNEBACTERIUM DIPHTHERIAE* ATCC NO. 296 [1]

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SUMMARY

Corynebacterium diphtheriae No. 296 was shown to have mean D values of 6.65, 1.68, 0.00547, 0.00314, 0.00140, and 0.00084 min. in milk at temperatures of 120, 125, 154, 156, 158, and 160° F., respectively. The mean D values for the organism in chocolate milk were 5.60, 1.18, 0.00758, 0.00556, 0.00407, and 0.00230 min. at respective temperatures of 120, 125, 148, 150, 152, and 154° F. Mean D values of 5.06, 0.72, 0.00309, 0.00238, and 0.00175 min. were found in cream for respective temperatures of 120, 125, 136, 138, and 140° F. In ice cream mix the organism was found to have mean D values of 15.0, 6.0, 0.00083, 0.00038, 0.00037, and 0.00027 min. for temperatures of 120, 125, 162, 164, 166, and 168° F., respectively.

According to the theory of logarithmic destruction of bacteria by heat, sterilization can not be achieved in a sample of infinite size. Therefore, for purposes of calculation, one must set arbitrary limits in order to determine a given process value. If one accepts a 10 D value process as being adequate for proper pasteurization, a holding time of 0.01 min. (0.6 sec.) at 160.5° F. will reduce the concentration of viable cells of *C. diphtheriae* No. 296 to the arbitrarily chosen lower limit of acceptability in milk and ice cream mix, which were found to provide more protection to the test organism than did chocolate milk and cream containing 40% fat.

Although a great deal of research has been done in the past to formulate the time-temperature relationships for pasteurization, the majority of the data available for *Corynebacterium diphtheriae* are such that it is difficult, at best, to apply them to conventional process calculations. Many of these studies on the thermal destruction of *C. diphtheriae* failed to report such pertinent variables as age and concentration of the culture, the growth medium, incubation temperature, sampling methods, and sample heating and cooling times. In addition, the authors agree with Ball (1), who stated that results such as those obtained under the above-mentioned conditions are of limited value in establishing thermal death curves.

The studies herein represent an attempt to determine the D[1] values for *C. diphtheriae* No. 296 when it was suspended in milk, chocolate milk, cream, or ice cream mix while being subjected to definite temperatures for specific exposure times. Accurate controls were kept on the age, incubation temperature, and inoculum concentration of the culture, as well as temperature of exposure, sampling and cooling times, and composition of the suspending fluids. It was

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[2] D value—the slope of the destruction rate curve or the time required to reduce a given bacterial population by 90% at a given temperature.

anticipated that the application of such controls would help eliminate the various anomalies presently existing in the literature.

REVIEW OF THE LITERATURE

Perhaps the earliest work concerning the thermal inactivation of *C. diphtheriae* was that reported by Brieger and Fraenkel (2), who in 1890 indicated that this organism was destroyed at 131° F. with a 45-min. heating and holding time. These results were confirmed 2 yr. later by Welch and Abbott (13). Loeffler (4) reported that *C. diphtheriae* was destroyed at 140° F. in 30 min. and Rosenau (9) stated that this organism was killed in milk heated to 131° F. and held at that temperature for 2 min. Using a commercial vat pasteurizer, Schorer and Rosenau (10) reported that *C. diphtheriae* was destroyed in milk when this liquid was heated to 140° F. and held for 2 min. The initial concentration of the test organism was not specified. Irwin (3) processed suspensions of *C. diphtheriae* in milk with the Electropure short-time pasteurizer and reported that a temperature of 140° F. destroyed this organism with holding times as short as 8 sec.

In 1956, Read, Boyd, Litsky, and Hankinson (6) reported the development of a small-bore, tubular heat exchanger in which milk or other fluids are heated by electrical resistance across the walls of the tube. Read *et al.* (7, 8) sterilized suspensions of *C. diphtheriae* in autoclaved milk (1×10^6 cells per milliliter), using mean temperatures of 161.4 and 157.4° F., with respective heating times of 0.25 and 0.50 sec. Using water bath immersion techniques, Oldenbusch, Frobisher, and Shrader (5) showed that the organism did not survive 145-150° F. for a 0.5-min. holding time when ice cream mix was used as the vehicle.

No data are known concerning the thermal resistance of this organism in chocolate milk or cream.

EXPERIMENTAL PROCEDURE

In order to construct adequate thermal death time curves it was decided to carry out these studies both at low temperatures with relatively long holding times and at high temperatures with short exposure times.

For the low temperature studies (120 and 125° F.) 396 ml. of test fluid was placed in a 500-ml. three-necked distilling flask. A standardized thermometer was placed into one of the necks. The other neck was used to introduce the bacterial suspension and to extract samples. The center port was used to seat a motor-driven stirring apparatus which agitated the suspension so as to provide a homogeneous suspension together with an even heat distribution in the flask. The flask was immersed in a water bath to a point where the water level in the bath was approximately 0.5 in. above that of the test fluid in the flask. Temperature was regulated by an electronic bath control unit and a microset mercury thermoregulator with an accuracy of $\pm$ 0.02° F. The water in the bath was agitated by continuous circulation to provide even temperature distribution. This apparatus is shown in Figure 1.

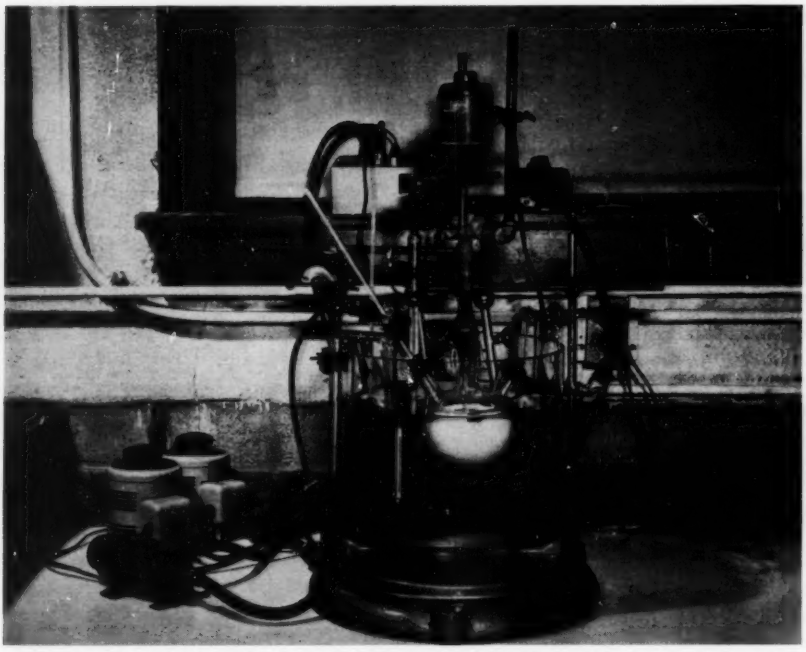

FIG. 1. Water bath used for *D* value determinations with relatively low heat exposure temperatures.

The culture of *C. diphtheriae* ATCC No. 296 (Park 8 strain) was used in a previous study done in this laboratory (8). The culture was maintained by daily transfer on slants of brain heart infusion agar (BHI), and incubated at 98.6° F. for 22-26 hr. The inoculum was harvested from the slants with sterile distilled water, filtered through sterile Reeve-Angel No. 235 filter paper to remove large clumps of cells, and standardized at 1×10^8 cells per milliliter with a nephelometer.

The suspending fluid (milk, chocolate milk, cream, or ice cream mix) was placed in the holding flask and brought to the desired temperature. Four milliliters of the standardized bacterial suspension was then added to the liquid in the flask. The addition of the 4.0 ml. of the bacterial inoculum was found to cause a temperature reduction of 0.5° F. To compensate for this, the suspending fluid in the flask was heated to 0.5° F. higher than that finally desired, so that the exact holding temperature would be obtained upon inoculation at zero time. The water bath temperature was also simultaneously lowered 0.5° F. by adding a predetermined amount of ice water and resetting the thermoregulator.

An automatic bulb pipette calibrated to remove 1.0 ml. was used to sample the bacterial suspensions of the heated test fluid. These aliquots were quickly

dispensed (3 to 5 sec.) into dilution blanks containing given amounts of sterile distilled water. Since the temperature rise upon the addition of the heated fluid never exceeded 3° F., cooling time in the dilution blanks was considered insignificant.

Bacterial concentrations after heating were determined by using pour plate methods with BHI agar as a recovery medium. Samples were plated within 10 min. after collection and incubated for 48 hr. at 98.6° F. prior to counting. Since the vehicles used in these studies were not sterile prior to inoculation, final counts were determined by counting only those colonies which were typical of the test organism.

The destruction rate curves were constructed by plotting the bacterial survivors on the logarithmic scale (ordinate) against the respective exposure time on the linear scale (abscissa) on semilogarithmic graph paper. A line of visual best fit was drawn which best satisfied the resulting points and the D value was taken as the slope of this line. The thermal death time curves for this organism were determined by plotting the mean D values obtained for each of the process temperatures used for a given test fluid on the logarithmic scale against the respective temperature on the linear scale of semilogarithmic graph paper.

Studies involving high temperatures were carried out in a tubular heat exchanger (Figure 2). The instrument is composed of a 5-gal. tank made of stainless steel, and a small-bore stainless steel tube made up of a preheating and a heating section. Heat is derived by heat exchange from the tube, which is heated by high amperage, low voltage alternating current. The fluid being tested is forced from the tank through the small-bore tube by air pressure at a rate so as to insure turbulent flow and, therefore, even heat distribution in the system. A more detailed description of the instrument has been reported by Read *et al.* (6).

In these studies, heating was accomplished by two sections of the tube. The first, designated as the preheating section, was used to raise the temperature of the test fluid from approximately 100° F. to a constant 120° F. The second, or the heating section, was used to raise the temperature of the fluid from 120° F. to the final heating temperature desired for the process.

The heating time was maintained at 0.25 sec., that is, it took exactly 0.25 sec. for the fluid to reach the desired temperature in passing through the heating section of the tube. A calculated holding time of 0.0113 sec., which was inherent in the process, was the time required for the test fluid to reach the surface of the collection diluent from the point of final heating.

The method used to prepare the bacterial inoculum was similar to that described for low-temperature investigations. Five gallons of the suspending fluid was placed in the holding tank of the instrument and mixed with 190 ml. of the prepared bacterial suspension. The mixture was stirred manually with a large stirring rod to insure homogeneity.

Unheated samples were collected at the beginning, midway, and at the end of an experimental run. The bacterial counts of these samples did not vary

FIG. 2. Tubular heat exchanger used for D value determination with relatively high exposure temperatures. (1) product reservoir, (2) ultraviolet hood and exhaust, (3) air pressure regulators, (4) preheating tube, (5) heating tube, (6) temperature recorder–preheating section, (7) temperature indicator–heating section, (8) sample collection hood, (9) variable voltage transformer–preheating section, (10) variable voltage transformer–heating section, and (11) one of two constant voltage transformers.

significantly. Samples processed in the heat exchanger were cooled by collection in pre-cooled, sterile distilled water. Since thermocouple measurements showed that temperatures in the collecting flasks during and immediately after collection never exceeded 100° F., cooling times were considered insignificant. Plating and plate count methods were identical to those described for the low-temperature studies.

Because the high-temperature studies involved processing with a set heating time to varying final temperatures, the D value could not be determined graphically as previously described. D values in this phase of the studies were determined mathematically, using the method of Stumbo, Murphy, and Cochran (11). The formula used is as follows:

$$Dn = \frac{U}{\log a - \log b}$$

Where U is the holding time at a given temperature n; a is the initial bacterial concentration; and b is the concentration of bacterial survivors after

exposure to temperature n for U time. Since the heat exchange apparatus based its effectiveness on the heating time to a given temperature, the total holding time (U) was consequently unknown and had to be determined by the conversion of heating time to holding time. It was necessary to calculate an individual holding time for each of the temperatures investigated with each of the menstrua. This was done by plotting a thermal death time curve from the results of the low-temperature investigations of the organism in the suspending fluid being studied. This curve was extrapolated in order to provide the theoretical D values of the organism at temperatures as high as the maximum process temperature employed. Lethal rates ($1/D$) of the organism were calculated from D values related to temperatures lying between the preheating and final heating temperatures. These temperatures were then related graphically to selected heating times ranging from 0.0 sec. for an initial heating temperature of 120° F., to 0.25 sec. for the final heating temperature. The heating time for intermediate temperatures was calculated by interpolation. This could be done since the heating characteristics in the heating tube were found to be linear.

The lethal rates corresponding to given heating temperatures were correlated to the heating times at which the particular temperatures were obtained.

A lethality plot, with arbitrary holding time, was then drawn which corresponded to the final heating temperature. The area under both the heating curve and the standard holding time curve was determined with a compensating polar planimeter. By establishing a ratio of total area to holding time between the two curves, the equivalent holding time (U) was calculated for the original heating time curve. This was performed for each final heating temperature and suspending fluid studied.

The suspending fluids studied were prepared as follows: (a) Raw milk was obtained from a bulk cooling tank at the University of Massachusetts Dairy. The D values for *C. diphtheriae* No. 296 in raw milk are given in Table 1.

TABLE 1

D values for *Corynebacterium diphtheriae* No. 296 in raw milk

Temperature (° F.)	No. of trials	Mean D (min.)	Range (min.)
120	4	6.65	6.0-7.0
125	5	1.68	1.2-2.1
154	5	0.00547	0.00436-0.00751
156	6	0.00314	0.00192-0.00608
158	6	0.00140	0.00081-0.00222
160	6	0.00084	0.00065-0.00131

(b) Chocolate milk was prepared by heating 396 ml. of raw milk to 100° F. and mixing it with 24.0 g. of granulated sugar and 4.8 g. powdered cocoa (Van Houten's Suma Dutch Process). The chocolate milk used in the high-temperature studies was prepared in larger quantities using the same formula. The D values for the organism in chocolate milk are shown in Table 2. (c) The

TABLE 2

D values for *Corynebacterium diphtheriae* No. 296 in chocolate milk

Temperature (° F.)	No. of trials	Mean D (min.)	Range (min.)
120	6	5.60	5.0-6.6
125	4	1.18	1.1-1.3
148	12	0.00758	0.00298-0.01512
150	12	0.00556	0.00313-0.01510
152	12	0.00407	0.00262-0.00654
154	8	0.00230	0.00058-0.00437

cream used was obtained by separating milk to a fat content of 40% by the Babcock test. Table 3 provides the *D* values for *C. diphtheriae* No. 296 in this product.

TABLE 3

D values for *Corynebacterium diphtheriae* No. 296 in raw cream

Temperature (° F.)	No. of trials	Mean D (min.)	Range (min.)
120	5	5.06	4.6-5.8
125	6	0.72	0.55-0.95
136	8	0.00309	0.00254-0.00345
138	8	0.00238	0.00194-0.00288
140	8	0.00175	0.00156-0.00194

(d) Ice cream mix was prepared as follows (based on 100 ml. of the product):

40% Raw cream	31.17 ml.
Skimmilk	48.42 ml.
Nonfat dry milk	5.06 g.
Granulated sugar	15.00 g.
Stabilizer	0.30 g.
Emulsifier	0.05 g.

In preparing this product for high-temperature heat exchange studies the ingredients were mixed while being heated to 100° F. Normally, much higher temperatures are used, but this was avoided so as to prevent overheating the product. Since the ice cream mix was not thoroughly heated during formulation, the emulsifier was not completely dissolved. Because the nature of the small-bore heat exchanger is such that it is unable to tolerate large particles, the mix was filtered through two double layers of cheese cloth prior to processing. The *D* values for *C. diphtheriae* No. 296 in ice cream mix are given in Table 4.

TABLE 4

D values for *Corynebacterium diphtheriae* No. 296 in ice cream mix

Temperature (° F.)	No. of trials	Mean D (min.)	Range (min.)
120	5	15.0	12.3-17.6
125	6	6.0	3.9- 7.1
162	8	0.00083	0.00066-0.00114
164	8	0.00038	0.00032-0.00048
166	8	0.00037	0.00031-0.00041
168	8	0.00027	0.00026-0.00030

DISCUSSION

It appears that the four suspending fluids offer varying amounts of protection to *C. diphtheriae* No. 296, wherein the organism shows the greatest amount of resistance in ice cream mix. Milk, chocolate milk, and cream offer decreasing amounts of protection in that order. If one uses these data to construct thermal death time (TDT) curves it is seen that the destruction rate is similar in ice cream mix, milk, and chocolate milk. When cream is used as a suspending fluid, the slope of the TDT curve indicates that this vehicle offers much less protection to this organism than do the other test fluids studied at temperatures of 136° F. and above.

The destruction rate curves for this organism when processed in each of the products at the lower temperatures studied (120 and 125° F.) were characterized by a slight lag in lethality in the initial phases of each determination. Thereafter, the death of this organism is found to follow very closely the logarithmic order. In all cases, the initial lag was ignored when a line of best fit was used to obtain the *D* value.

Since the dairy industry, in the pasteurization of milk, is primarily concerned with process values rather than *D* values, it is of interest to attempt to express these results in terms of the former. If one accepts the theory that bacteria follow a logarithmic order of death when exposed to lethal heat, it follows that one can not completely eliminate a given population of a given bacterium in a sample of infinite size. Because of this, one must establish arbitrary standards for the pasteurization of milk products. It is felt that with our present production practices it would be highly improbable that one would receive raw milk, which is otherwise satisfactory, with a population exceeding 1,000 cells per milliliter of *C. diphtheriae*.

To convert *D* values to process values, one must establish maximum limits of acceptability for pasteurization. It would seem that establishing this limit at one viable cell of *C. diphtheriae* per 10^7 ml. of milk would not be unreasonable. With these criteria in mind the process value is equivalent to 10 *D* values. The temperatures required for obtaining a total process equivalent to 10 *D* values with *C. diphtheriae* No. 296 for five selected holding times with the four vehicles studied are shown in Table 5. It is seen that with a 0.01 min. (0.6 sec.) holding time a holding temperature of 161.5° F. is adequate for a satisfactory process with *C. diphtheriae* No. 296 if the 10 *D* criterion is accepted.

TABLE 5

Temperature required to reduce *Corynebacterium diphtheriae* No. 296 through 10 D values for five selected holding times

Holding time *(min.)*	5.0	1.0	0.25	0.1	0.01
			(° F.)		
Milk	131.6	139.2	146.0	150.0	160.5
Chocolate milk	130.3	137.5	143.5	147.8	153.0
Cream	125.5	129.2	132.4	134.7	140.1[a]
Ice cream mix	135.0	141.7	147.6	151.5	161.3

[a] Extrapolated value.

These data also show that *C. diphtheriae* No. 296 can be eliminated from milk by using a holding time considerably less than that applied in the HTST method of commercial pasteurization.

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A MANUFACTURING METHOD FOR IMPROVING THE PHYSICAL CHARACTERISTICS OF WINTER BUTTER[1, 2]

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SUMMARY

Two methods of manufacturing butter were studied. One, the control procedure, consisted of cooling the cream after pasteurization to 46° F. The cream was held at that temperature overnight (15 hr.) and churned the next morning. The other method, the cream-temperature treatment or experimental method, consisted of cooling the cream after pasteurization to 46° F. and holding it at that temperature for 2 hr. Then it was heated slowly to 66° F. (heating time 1 hr.) with water in the jacket of the vat at 79° F. This temperature was maintained for 6 hr. The cream was then cooled to 61° F., held overnight (15 hr.), and churned the next morning.

Examination of the butter made by the two methods, when at a temperature of 48° F., showed that the cream-temperature treatment resulted in butter that was relatively soft and waxy and possessed smooth spreading properties. The butter made by the control procedure was generally hard, crumbly, and sticky and had poor spreading properties. Chilled wash water (40° F. or lower) and low-temperature storage (below 0° F.) were also found to be beneficial in the attainment and maintenance of desirable body characteristics in butter.

The manufacture of butter with desirable physical characteristics has long been a problem of the butter industry. Butter graders and distributors throughout the country report that much of the butter made possesses undesirable physical properties. These undesirable characteristics vary with the section of the country and the season of the year in which the butter is produced.

Previous investigators (1, 3, 7, 10) have indicated that changes in the composition of the milk fat are responsible for the majority of the variations in the physical characteristics of commercially manufactured butter. Dolby (3) has estimated that 80% of the variation is due to changes in the composition of the milk fat. The remaining variation can be attributed to the method of manufacture. Changes in the composition of the milk fat are due to the varying types of feed fed to dairy cows during the different seasons of the year. Since these can not be changed materially because of economic reasons, it appears that the immediate and practical solution to the problem lies in a modification of the manufacturing practices.

Modified manufacturing methods have been recommended by previous investigators which in part eliminate some of the undesirable physical characteristics, but do not as a whole markedly improve the spreading property. Richardson and Abbott (7) recommended a prechurning heat treatment to overcome

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defects caused by the feeding of alfalfa hay. Coulter and Combs (1) suggested that to overcome the body and texture defects of winter-made butter, excessive cooling of the cream after pasteurization should be avoided. The use of cream storage temperatures above 50° F. has been recommended by some investigators (3, 6, 9) to help eliminate the undesirable physical properties of winter-made butter. Several investigators (1, 2, 6, 8, 9, 11) have suggested the use of cold wash water (below 45°) for washing and tempering the butter granules before working as a means of reducing the hardness of butter made during the winter months.

Research workers in Denmark for a number of years have studied butter manufacturing methods during summer and winter for the purpose of developing butter with uniform physical characteristics throughout the year (4, 5). They have, on the basis of their work, recommended manufacturing methods for the production of winter and summer-made butter with uniform physical characteristics.

The purpose of this investigation was to try to eliminate undesirable physical characteristics of winter-made butter when manufactured under Oregon conditions. One of the Danish methods for the manufacture of butter during the winter months was modified for this study.

EXPERIMENTAL PROCEDURES

Methods used. Cream used in this study was obtained from milk produced during the fall, winter, and early spring months in the Willamette Valley. Iodine values of the cream ranged from a low of 29.65 in March to a high of 41.40 in April. Melting point values ranged from a high of 95° F. in January to a low of 89.9° F. in April. Nine churnings were made from farm-separated cream and 63 were made from plant-separated cream. Duplication of commercial practices was attempted in all cases. Cream was generally received and churned once each week. Usually, only one method of manufacture was used each week. On four occasions split churnings were made. Two vat pasteurizers, one 2,500-lb. capacity, the other 850-lb. capacity, were used to pasteurize and temperature-treat the cream. Both were equipped with manual temperature controls for the treatment involved in the temperature-treating procedure. A 1,000-lb.-capacity wooden roll-less churn and a 400-lb.-capacity wooden roll-less churn were used to churn the cream. Cold storage rooms maintained at −10, 0, 40, and 48° F. were used for storing and tempering the butter before examination.

Cream to be churned was pasteurized at 155-160° F. and held at this temperature for 30 min. Cream for the control churnings was cooled immediately to 46° F. (cooling time usually 2 hr.), held at that temperature overnight, and churned the next morning. Cream for the experimental churning was cooled to 46° F. after pasteurization (cooling time usually 2 hr.) and held at that temperature 2 hr. Then it was heated slowly to 66° F. (heating time 1 hr.) with the water in the jacket of the vat at 79° F. This temperature was maintained for 6 hr. The cream was then cooled to 61° F. and held overnight. The next morning the cream was cooled to churning temperature and churned immediately.

Churning procedures were the same for both the control and experimental churnings. The cream was pumped into the churn, churned, the granules washed, and worked in the usual manner. The only variation used was wash water temperature.

Sampling procedure. Two pounds of butter were removed from the churn upon completion of the working procedure. These were molded into 1-lb. prints in a wooden mold and wrapped in parchment paper. One pound was placed in each of two refrigerated rooms maintained at −10 and 40° F. The sample was stored for 2 wk. The remaining butter was placed in 68-lb. parchment-lined wire baskets and stored at 40° F. for 48 hr. before being printed with a screw-type printer. Two 1-lb. prints were obtained after printing and wrapped in parchment paper. They also were stored at −10 and 40° F., respectively, for 2 wk. All the samples were tempered to 48° F. for 72 hr. before being examined for physical characteristics.

Methods of examining physical characteristics. All experimental samples of butter obtained were examined for body, texture, hardness, and spreading property. A stainless steel table knife was used to cut and spread the butter on a wooden board. The spreading property was noted as poor, fair, good, or excellent. Any body and texture defects such as crumbliness and stickiness also were observed at this time.

A hardness tester similar to the one used by the Danish investigators (4, 5) was designed and constructed by the National Appliance Company, Portland, Oregon. The tester consisted of a constant speed motor which forced a flat, round plunger with a surface area of 4 cm.^{2} down into the butter at a rate of 20 mm. per minute. A scale placed below the plunger measured the hardness in kilograms. Hardness was expressed in kilograms and was determined in a room maintained at 48° F. Duplicate determinations were run on all samples and the hardness was considered to be the average of the two. In the majority of the samples, the duplicates were identical.

RESULTS AND DISCUSSION

The cream in the experimental churning tended to churn faster, with a greater loss of fat than the cream in the control churning; therefore, it was necessary to use a churning temperature three to four degrees lower for the experimental churnings. This was demonstrated in the split churnings that were made. Cream from the same source was used; a portion was used with the control procedure and the remainder was temperature-treated. In each case, the temperature-treated cream churned in almost the same time but at a temperature three to four degrees lower. This is shown in Table 1.

During the course of this investigation, 40 churnings were manufactured using the control procedure. Butter from these 40 churnings was criticized for excessive hardness, crumbliness, stickiness, and poor spreadability. The use of wash water below 45° F. did not appear to improve the physical characteristics of the butter made by this procedure.

TABLE 1

Effect of the cream-temperature treatment on churning time and temperature

Churnings	Date manufactured	Churning temp.	Churning time
		(° F.)	*(min.)*
Experimental	12-12-56	48	25
Control	12-12-56	52	35
Experimental	1-10-57	48	35
Control	1-10-57	52	35
Experimental	2-21-57	47	55
Control	2-21-57	50	65
Experimental	4-25-57	47	30
Control	4-25-57	50	35

A total of 32 churnings of butter was made during this study, using the cream-temperature treatment. In general, the butter manufactured when the cream treatment was used possessed superior physical characteristics to butter made with the control procedure. Not a single experimental churning received a poor rating on spreading property.

Hardness. Since there was not an equal number of control and experimental churnings (40 control and 32 experimental), 18 control and 18 experimental churnings were randomly selected and a statistical analysis was made on their hardness values. Statistical comparisons were made on the effects of (a) control and experimental methods of manufacture and (b) printing the butter immediately after churning and printing with a screw-type printer after the butter had become firm. Results of the analyses are presented in Table 2.

The analysis of variance of these data indicated that the cream-temperature treatment resulted in a softer-bodied butter than the control procedure. It also showed that printing with a screw-type printer after the butter had become firm lessened the hardness values of the butter. This is illustrated by the sampling time means given in Table 2. The mean hardness value for butter that was printed immediately upon completion of working was 2.81; whereas, the mean

TABLE 2

Analysis of variance of hardness values

Source variation	d.f.	Sum of squares	Mean square	F
Between treatments	1	.2813	.2813**	20.68
Between sampling times	1	.0613	.0613**	7.96
Interaction between treatments and sampling times	1	.0012	.0012	.16
Among churnings within treatments	34	.4636	.0136	
Interaction between sampling times and churnings within treatments	34	.2625	.0077	

** Significant at a probability level of 0.01.

Treatment means

Experimental = 2.71 ± .019

Control = 2.83 ± .019.

Sampling time means

After printing at completion of working = 2.81 ± .015

After printing with screw-type printer after 48 hr. of storage at 40° F. = 2.75 ± .015.

hardness value for the butter that was printed with a screw-type printer after the butter had become firm (stored at 40° F. for 48 hr.) was 2.75. This difference is significant at a 1% probability level. Since the interaction between treatments and sampling times was not significant, this indicated that the difference between experimental and control churnings is the same at both sampling times.

Storage time and temperature. Butter was examined for physical characteristics throughout this investigation when at a temperature of 48° F. It was believed that 48° F. would be typical for household refrigerators and that butter examined at this temperature would represent consumer use conditions. Therefore, if butter had desirable spreading properties at 48° F., it would be acceptable to the consumers.

To determine the effect of storage temperature on the physical characteristics of the control and experimental butter, samples from both were stored at 40, 48, 60, and 70° F. for 14 days. The samples from the respective storage temperatures were examined at the end of seven and 14 days after being tempered at 48° F. The data are given in Table 3.

No change was noticed in the physical properties and flavor of the experimental butter when stored at 40° F. for 14 days. During the first seven days of storage at 48° F., no apparent change took place in the body and texture of the butter, but after 14 days the butter became firmer and lost its desirable spreading property. Changes in the physical characteristics of the butter stored at 60 and 70° F. were very noticeable after seven days of storage. After 14

TABLE 3

Effect of storage temperature on the physical characteristics

Experimental butter

Storage ° F. temp.	Into storage		7 days		14 days	
	Flavor score	Physical characteristics	Flavor score	Physical characteristics	Flavor score	Physical characteristics
42	93	Excellent	93	Excellent	92	Excellent
48	93	Excellent	93	Excellent	92	Good
60	93	Excellent	91	Sticky	91	Hard, brittle
70	93	Excellent	89	Hard, brittle, crumbly	88	Very hard, crumbly, brittle

Control butter

Storage ° F. temp.	Into storage		7 days		14 days	
	Flavor score	Physical characteristics	Flavor score	Physical characteristics	Flavor score	Physical characteristics
40	93	Hard, crumbly, sticky	92	Hard, crumbly, sticky	92	Hard, crumbly, sticky
42	93	Hard, crumbly, sticky	92	Hard, crumbly, sticky	92	Hard, crumbly, sticky
60	93	Brittle, sticky	91	Hard, crumbly, sticky	89	Hard, crumbly, sticky
70	93	Hard, crumbly, sticky	89	Hard, crumbly, sticky	87	Very hard, brittle, very crumbly

days, the butter had lost completely its smooth spreading property and was hard, brittle, and crumbly. This change in body and texture was also noted in butter that had been exposed to room temperature for a few hours and recooled to 48° F.

Storage temperature effects were similar for the butter from the control churnings. At 60 and 70° F. the poor physical properties became more pronounced. Changes in flavor score of butter from both methods of manufacture were identical.

To determine the effects of long-time storage at −10° F. on the physical properties of the control and experimental butter, samples were randomly selected and examined at 48° F. after having been stored for different periods of time at −10° F. Very little change was noted in the physical characteristics of either the experimental or the control butter during the low-temperature storage. It was significant that the excellent spreading property of the butter from the experimental churnings was not lost during low-temperature storage.

It should be noted that during the course of this investigation it was possible to manufacture butter with excellent physical properties, using the cream-temperature treatment when the iodine value and the melting point value indicated relatively hard fat. It was also possible to manufacture butter that was hard, crumbly, and spread poorly with the control procedure when the iodine value and the melting point value indicated relatively soft fat.

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COMPARISON OF ACID AND NONACID VOLUMETRIC METHODS FOR DETERMINING THE PERCENTAGE OF BUTTERFAT IN RAW MILK[1]

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SUMMARY

Raw milk of varied fat content was comparatively analyzed by the Gerber, Dairy Products Section (DPS), TeSa, Schain, Babcock, and Mojonnier procedures. The Babcock, Gerber, and DPS averaged higher than the Mojonnier: .054, .077, and .003% butterfat, respectively, whereas the TeSa and Schain were lower, .064 and .053%. All of the above differences, except that for the DPS, were statistically significant ($P < 0.01$). When compared with the Babcock, the Gerber test was slightly higher (.023%), while the DPS, TeSa, and Schain tests were lower: .038, .082, and .044%, respectively. Only the TeSa and Schain differences were statistically significant. The Gerber, DPS, TeSa, and Schain tests were more closely correlated with the Babcock than they were with the Mojonnier.

More Gerber tests agreed within $\pm$ 0.1% of the Babcock (93.9%) than did those of the other methods, DPS (86.1%), TeSa (72.2%), and Schain (57.2%). The TeSa test showed more tester variation than did the other methods studied.

As the test of the milk increased from 2.5 to 7.0% fat, the differences obtained by the Gerber and DPS were increasingly higher; whereas, those obtained by the TeSa and Schain methods were increasingly lower than the Mojonnier. The Babcock did not show a similar nomogram effect compared to the Mojonnier.

An accurate analysis for butterfat in milk has been of great importance to the dairy industry for many years. The Roese-Gottlieb or Mojonnier (ether extraction) methods are accepted as the most accurate procedures for this determination. However, due to their relative expense in time, labor, and equipment the volumetric Babcock test, which is much faster, cheaper, and simpler to conduct, is relied upon by the dairy industry in the United States for routine analyses. In most other countries, the Gerber test, which is quite similar to, but faster than, the Babcock test is used. The Gerber test was adopted as a legal procedure for use in dairy plants in Pennsylvania in February, 1959. It also is used in dairy plants in New York, New Jersey, California, and Florida. Although many workers (3, 5, 8, 24, 37) have compared the Babcock and Gerber procedures, it was deemed desirable to conduct another comparison using more up-to-date equipment than was available when most of the earlier investigations were made.

In recent years, the Schain (33, 34), Dairy Products Section (DPS) (30, 31, 32), and TeSa (18, 19) tests have been developed to determine the percentage of butterfat in milk and other dairy products. These tests employ detergents and other reagents in lieu of strong sulfuric acid, as is required in both the Babcock and Gerber procedures. The Schain and TeSa tests also

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dispense with centrifugation, which offers another advantage over the acid procedures. The TeSa test is the most recent of the three new tests and considerable interest has been shown in its possible use in D.H.I.A. programs (28) as well as in other areas of testing.

Although the Schain (6, 9, 10, 13, 17, 20) and DPS (15, 17, 32) tests have received rather careful investigation, the TeSa procedure has not undergone as extensive study.

EXPERIMENTAL DESIGN

This study was designed to compare the accuracy of the Gerber, DPS, Schain, and TeSa tests with the Babcock method and with the Mojonnier ether extraction procedure for determination of butterfat in milks of various fat percentages. Raw milk of approximately 3.8% fat was adjusted with either raw skimmilk or cream (36.0% fat) to provide milks testing 2.5 to 7.0% butterfat at 0.5%, $\pm$ 0.2%, intervals. The milks were checked for composition by the Babcock test. Thus, ten samples of milk were included in each of nine replicates conducted by two carefully trained technicians. Each technician made up his own lots of milk and conducted each test in duplicate. Only one of the technicians included the Mojonnier procedure in his comparison.

All equipment was checked for accuracy of calibration. Reagents were fresh and of good quality. Schain reagent was obtained from Merck and Company, Inc., Rahway, New Jersey, and the procedure accompanying the reagent was followed. The directions of Sager *et al.* (32) were followed for the DPS test. The procedure recommended by Technical Industries, Inc., Fort Lauderdale, Florida (36), was used for the TeSa test. The directions as outlined by the Milk Industry Foundation (26) were used for the Gerber method. The procedure for the Babcock test was taken directly from official methods (1) and the Mojonnier test followed the directions published by the company (27).

Prior to testing, each milk sample was warmed to 100° F. and held at this temperature for 5 min., tempered to 60 to 70° F., and mixed thoroughly by pouring back and forth at least six times before pipetting. All reagents also were tempered to 60 to 70° F. before adding them to the milk sample.

Statistical procedures used in this study were those outlined by Snedecor (35).

RESULTS

Since the Babcock has been the basis for payment to the farmer for milk for about 70 yr., it would appear reasonable to expect other volumetric tests to compare more closely to this test than to ether extraction procedures. Therefore, the data are presented first as a comparison with the Babcock test.

Table 1 compares the average per cent fat and average deviation of the four volumetric tests with the Babcock test. The standard error for the average deviation also is shown. Both technicians had similar results with the Gerber and DPS tests; however, Technician B showed considerably more deviation with the TeSa and Schain tests than did A. The average deviation from the Babcock test was less than 0.1% in all cases except for the TeSa and Schain

TABLE 1
Comparison of four methods of determining butterfat in milk with the Babcock test
(Average per cent fat of 90 tests in duplicate)

Technician	Babcock	Method			
		Gerber	D.P.S.	TeSa	Schain
A	4.716	4.739	4.691	4.671	4.735
		.049 ± .035[a]	.062 ± .044	.058 ± .046	.090 ± .063
B	4.764	4.787	4.713	4.646	4.657
		.050 ± .043	.072 ± .069	.126 ± .109	.149 ± .124
Average	4.740	4.763	4.702	4.658	4.696
		.049 ± .040	.067 ± .058	.092 ± .091	.120 ± .102

[a] Average deviation from the Babcock and its standard error.

tests for Technician B. These results indicate that the TeSa and Schain procedures are subject to more tester variation than the Gerber and DPS.

Table 2 shows a summary of the deviations from the Babcock procedure. On the basis of the percentage of samples within ± 0.1% of the Babcock test, closest agreement was obtained with the Gerber (93.9%), DPS, (86.1%), TeSa (72.2%), and Schain (57.2%), respectively. The majority of samples in the case of the TeSa, DPS, and Schain procedures were lower than the Babcock test (80.0, 68.3, and 50.6%, respectively), whereas the reverse was true with the Gerber procedure (56.1%). With the TeSa test, 27.8% of the samples were more than 0.1% lower than the Babcock, whereas 32.2% of the Schain tests were in this category.

Analyses of variance of the data by each technician are shown in Table 3. Almost identical analyses, with the exception of the degree of significance of methods, were obtained in each case. Based on the least significant difference between means, the TeSa test was significantly ($P < 0.05$) lower than the Babcock test for Technician A and highly significantly ($P < 0.01$) lower for Technician B. The Schain test also was highly significantly ($P < 0.01$) lower than the Babcock for B. The remaining differences among methods were not statistically significant.

The highly significant first-order interactions of methods × replicates (M × R) and levels × replicates (L × R) obtained in both cases are difficult to

TABLE 2
Deviations from the Babcock test of four methods of determining butterfat in milk by two technicians

Deviation	Method							
	Gerber		D.P.S.		TeSa		Schain	
	(No.)	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
Over + .10	10	(5.6)	5	(2.8)	0	(0.0)	19	(10.6)
+ .10	41	(22.8)	14	(7.8)	5	(2.8)	40	(22.2)
+ .05	50	(27.8)	18	(10.0)	16	(8.9)	29	(16.1)
.00	26	(14.4)	20	(11.1)	15	(8.3)	1	(0.6)
− .05	45	(25.0)	59	(32.8)	56	(31.1)	20	(11.1)
− .10	7	(3.9)	44	(24.4)	38	(21.1)	13	(7.2)
Over − .10	1	(0.6)	20	(11.1)	50	(27.8)	58	(32.2)
Total within ± .10	169	(93.9)	155	(86.1)	130	(72.2)	103	(57.2)

TABLE 3
Analyses of variance of five volumetric methods of determining butterfat in milk by two technicians

Source of variation	Degrees of freedom	Mean square	
		Technician A	Technician B
Total	899	1.9602	1.9880
Methods (M)	4	0.1569[b]	0.7118[a]
Level of fat (L)	9	194.0687[a]	196.2659[a]
Replicates (R)	8	0.7307[a]	0.8892[a]
Duplicates (D)	1	0.0004	0.9274
Remainder	877	0.0104	0.0121
First-order interactions			
M × L	36	0.0487[a]	0.0649[a]
M × R	32	0.0134[a]	0.0534[a]
M × D	4	0.0009	0.0035
L × R	72	0.0785[a]	0.0415[a]
L × D	9	0.0008	0.0024
R × D	8	0.0023	0.0045
Remainder	716	0.0018	0.0049
Second-order interactions			
M × L × R	288	0.0032[a]	0.0087[a]
M × L × D	36	0.0009	0.0014
M × R × D	32	0.0010	0.0017
L × R × D	72	0.0008	0.0027
Remainder	288	0.0008	0.0025

[a] Significant at 1% level of probability.
[b] Significant at 5% level of probability.

explain. The explanation offered by Hoover *et al.* (17) that "the conditions and manipulations of the Babcock test have been more highly standardized, through long study and use, than have those of the newer tests" also may apply here.

The highly significant interaction of methods × levels (M × L), also obtained in both cases, can be elucidated by an examination of Figure 1. As the percentage butterfat increased, the deviation of the Gerber test from the Babcock test, which was used as the standard, increased from an average of .024% lower at 2.5% fat to approximately .07% higher at 6.0 to 7.0% fat. The DPS test also increased from approximately .08% lower at 2.5% fat to approximately the same reading as the Babcock at 6.0 to 7.0% fat. The TeSa test was consistently lower (.05 to .11%) than the Babcock at the various levels of fat. Although a nomogram was used to correct the Schain readings, the results of this study indicate that additional correction factors are needed for close agreement with the Babcock test. The Schain test was .08% higher than the Babcock at 2.5% fat to approximately .22% lower at 7.0% fat. This was almost a straight-line decrease.

The correlation values for the four volumetric tests versus the Babcock and the standard errors of estimate based on a linear regression equation are shown in Table 4. Any test that has progressed to the point of being deemed worthy of comparison with the long-established Babcock and Gerber tests should be expected to show a high correlation value against either of these tests. The lowest correlation obtained was .9960 for the Schain test, whereas the highest

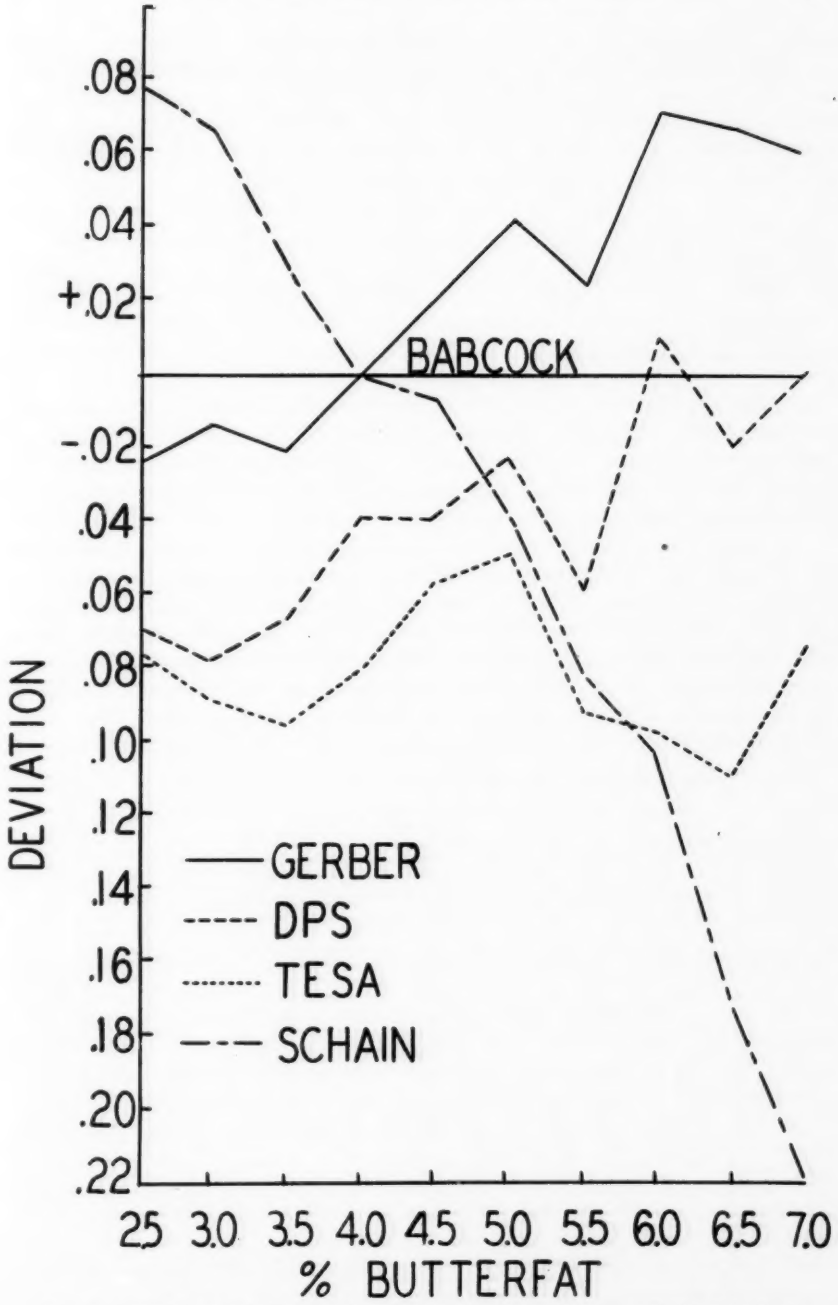

FIG. 1. Average deviations from the Babcock procedure of four methods of testing raw milk for percentage of butterfat.

TABLE 4

Correlations and standard errors of estimate of four methods of determining butterfat in milk with the Babcock test

Technician	Method			
	Gerber	D.P.S.	TeSa	Schain
A	.9996[a]	.9989	.9994	.9981
	.035[b]	.062	.057	.077
B	.9992	.9984	.9968	.9960
	.058	.081	.113	.120
Average	.9994	.9986	.9975	.9960
	.043	.084	.103	.121

[a] Correlation value.
[b] Standard error of estimate.

was .9996 for the Gerber test. Since all values are high, the maximum difference of .0036 in this case probably is significant. The standard errors of estimate show fairly close agreement with the standard errors of the average deviation in Table 1. These values also show that Technician B had more deviation from the Babcock with all four methods than did A.

A comparison of the five volumetric methods with the Mojonnier procedure by Technician B is presented in Table 5. Based on average deviations, the Babcock, Gerber, and DPS tests appear to give similar results, whereas both the TeSa and Schain tests show considerably more deviation from the Mojonnier test. However, on the basis of the summary of deviations of each test from the Mojonnier (Table 5), both the Babcock and DPS methods had 66.7% of the samples within ± 0.1% of the Mojonnier reading, the TeSa, 63.3%; the Gerber, 58.9%; and the Schain, 41.1%. With the Babcock, Gerber, and DPS, the majority of samples were higher than the Mojonnier, 78.9, 84.4, and

TABLE 5

Comparison of five volumetric methods of determining butterfat in milk with the Mojonnier procedure

(Average per cent fat of 90 tests in duplicate)

Deviation	Mojonnier	Method									
		Babcock		Gerber		D.P.S.		TeSa		Schain	
	4.710	4.764		4.787		4.713		4.646		4.657	
		.080 ± .050[a]		.087 ± .060		.085 ± .076		.106 ± .112		.149 ± .115	
		Deviations from Mojonnier									
		(No.)	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
Over +.10		26	(28.9)	37	(41.1)	16	(17.8)	6	(6.7)	20	(22.2)
+.10		26	(28.9)	18	(20.0)	15	(16.7)	7	(7.8)	17	(18.9)
+.05		19	(21.1)	21	(23.3)	19	(21.1)	16	(17.8)	8	(8.9)
.00		1	(1.1)	1	(1.1)	1	(1.1)	1	(1.1)	0	(0.0)
−.05		8	(8.9)	10	(11.1)	18	(20.0)	19	(21.1)	8	(8.9)
−.10		6	(6.7)	3	(3.3)	7	(7.8)	14	(15.6)	4	(4.4)
Over −.10		4	(4.4)	0	(0.0)	14	(15.6)	27	(30.0)	33	(36.7)
Total within ± .10		60	(66.7)	53	(58.9)	60	(66.7)	57	(63.3)	37	(41.1)

[a] Average deviation and its standard error.

55.6%, respectively. The TeSa test showed 66.7% of the samples with lower readings than the Mojonnier, whereas the Schain had an exact 50-50 distribution. The TeSa and Schain tests had 30.0 and 36.7%, respectively, of the readings lower than 0.1% of the corresponding Mojonnier reading, whereas the Babcock and Gerber tests had 28.9 and 41.1% of the samples with readings more than 0.1% higher than the Mojonnier reading. The DPS test had 17.8% more than 0.1% higher and 15.6% more than 0.1% lower.

The correlation values and standard errors of estimate based on a linear regression equation for the five volumetric tests versus the Mojonnier were: Babcock, .9985, .078; Gerber, .9989, .066; DPS, .9970, .118; TeSa, .9953, .135; and Schain, .9942, .142. Comparing these values with those in Table 4 for Technician B, it can be seen that the Gerber, DPS, TeSa, and Schain tests are more closely correlated with the Babcock test than they are with the Mojonnier.

Analysis of variance of the data revealed that both the Babcock and Gerber gave readings highly significantly ($P < 0.01$) greater than the corresponding Mojonnier reading, while the TeSa and Schain were highly significantly ($P < 0.01$) lower. The difference between the DPS and Mojonnier was not statistically significant.

The average deviation of the five volumetric tests from the Mojonnier at each level of butterfat is shown in Figure 2. The Gerber and DPS tests show an increasing slope with increasing fat, while the TeSa and Schain show a decreasing slope. The Babcock is consistently higher than the Mojonnier, presenting a fairly straight line from 2.5 to 7.0% fat.

DISCUSSION

For simplicity, the five volumetric tests will be discussed separately.

Babcock. The results of the present study agree with those of other investigations (2, 4, 7, 8, 12, 14, 25, 29), in that the Babcock gives results approximately .05 to .08% higher than ether extraction procedures. However, the extent to which the Babcock gave higher values than the Mojonnier did not increase with increasing fat test of the milk as reported by Hileman *et al.* (16). An explanation and suggestions for correction of this error has been given by Herreid (15).

Gerber. The advantages in speed and simplicity of the Gerber test as compared to the Babcock have been pointed out by Levowitz (21, 22, 23) and Gould (11, 12). The present study showed that the Gerber test is more highly correlated with the Babcock ($r = .9994$) and with the Mojonnier ($r = .9989$) than other tests studied. It averaged .023% higher than the Babcock and .077% higher than the Mojonnier. The higher values than the Babcock and ether extraction procedure agree very closely with other workers (8, 24, 37). The extent to which the Gerber gave higher results than the Babcock or Mojonnier increased as the milk became richer in fat. This agrees with the observation made by van den Burg *et al.*, as cited by Bakalor (3), on limited numbers of samples. At 3.5 to 4.5% fat, which would fit the majority of milk samples in

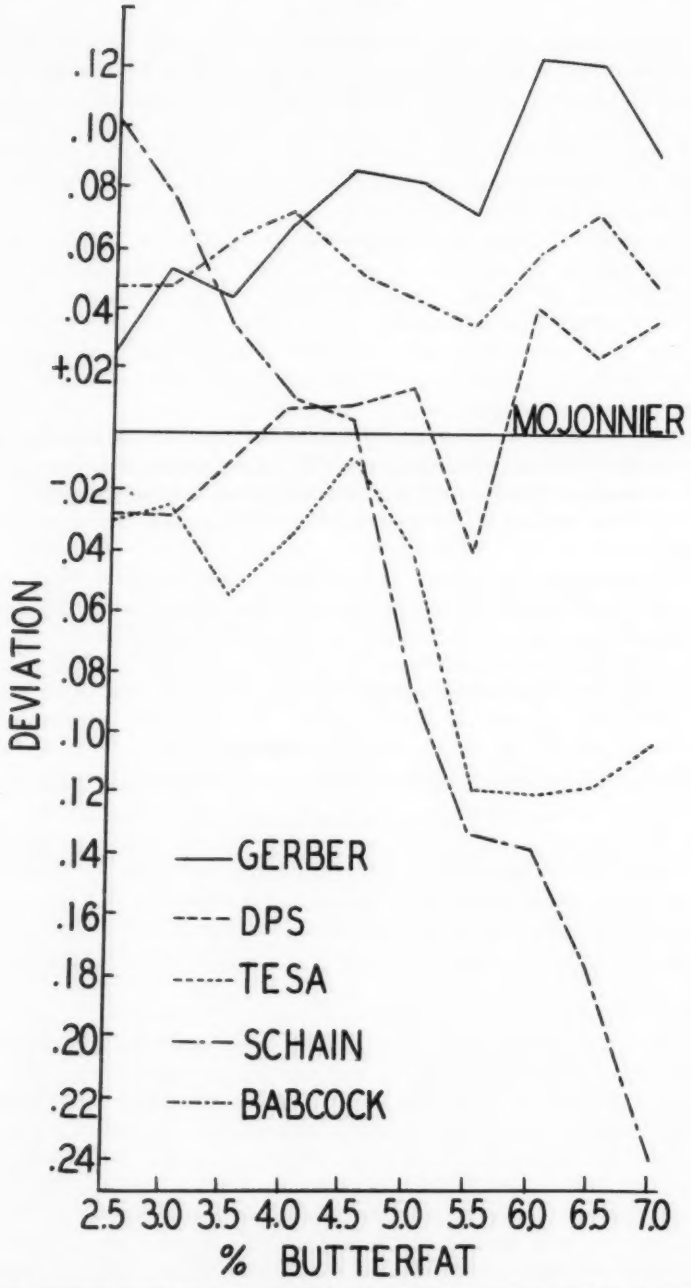

FIG. 2. Average deviations from the Mojonnier procedure of five methods of testing raw milk for percentage of butterfat.

dairy plants, the Gerber test showed less than .02% variation from the Babcock. With milks testing more than 6.0% fat, the Gerber test showed readings greater than .05% of the Babcock. On the basis of the present study, the Gerber test gives results comparable in accuracy to the Babcock for use in dairy plants where the range would not be expected in most cases to exceed 3.5 to 4.5% butterfat. However, it appears to be less accurate than the Babcock when the fat test exceeds 6.0%.

DPS. In general, of the three nonacid volumetric tests studied, the DPS compared most favorably with both the Babcock and Mojonnier procedures. However, it was subject to somewhat more variation than the Babcock or Gerber. The DPS showed a trend similar to that observed for the Gerber test with increasing richness of the milk. As pointed out by Hoover *et al.* (17), the DPS test requires more equipment and is more complicated to perform than the Schain test. This also is the case when compared with any of the volumetric tests in this study. With milks testing higher than 5.0 to 6.0% fat, a cloudy mass of material at the bottom of the fat column was frequently encountered. It was necessary to include this material when reading the test. By increasing the amount of reagent from the recommended amount of 5 to 7 or 10 ml., as suggested by Herreid (15), this difficulty was not encountered. Even though the DPS test eliminates strong sulfuric acid, it still requires a centrifuge and is much more tedious to perform than any of the five volumetric tests studied.

TeSa. A summary of previous studies of the TeSa test may be found in a report by Technical Industries, Inc. (36) and by Washburn (38, 39). The present study is not in close agreement with these results. The TeSa test was the only one of four volumetric tests studied which showed a statistically significant difference from the Babcock by each of the two technicians. Also, each technician obtained a different type of variation based on the richness of the milk. One showed a maximum deviation from the Babcock at 2.5 to 3.0% fat approaching practically zero deviation at 7.0% fat. The other technician showed just the opposite pattern, obtaining a maximum deviation at 6.5 to 7.0% fat with the smallest deviation at 2.5 to 3.0% fat. Since both technicians obtained quite similar results with the Gerber, DPS, and Schain procedures, the discrepancy with the TeSa is even more significant. It appears that the TeSa test is subject to more tester variation than other procedures. With the exception of the Schain test, the TeSa procedure was least significantly correlated with either the Babcock or the Mojonnier tests. Eighty per cent of the observations with the TeSa test were lower than the corresponding Babcock readings, with more than one-fourth of the readings being more than 0.1% lower. The average error was −.082%, which for all practical purposes may be interpreted as 0.1% on the TeSa bottle. Since D.H.I.A. and Official testing programs have relied on the Babcock for years, any new test used in these programs should give comparable results. It should not show a tendency to be generally lower than the Babcock or the Mojonnier tests.

The average error of −.082% (two technicians) compared with the Babcock would appear to make the TeSa test agree more closely with the Mojonnier than would the Babcock test. However, the one technician who included the

Mojonnier analysis in his study had an average error of −.118% with the TeSa vs. the Babcock and −.064% with the TeSa vs. the Mojonnier. Even if the TeSa did agree more closely with the Mojonnier than does the Babcock, it is questionable whether a penalty of approximately .08% fat should be imposed on dairy farmers who have relied on the Babcock test for many years. With further refinement and the development of a premixed reagent ready for use, the TeSa test shows excellent possibilities for use in the dairy industry.

Schain. The Schain test probably is the simplest to perform of all tests studied, and gives a very clear, sharply defined fat column. However, it makes use of a nomogram and, thus, is highly empirical. As pointed out by Bakalor (3), the application of nomograms to other tests also would give better agreement with ether extraction methods. The results of the present study show that additional correction factors are needed to obtain close agreement with either the Babcock or Mojonnier tests at the extreme levels of fat content. The Schain showed greater than .75% variation from both the Babcock and Mojonnier below 3.0% fat and above 5.5% fat. It showed the greatest average error and lowest correlation values of any of the tests.

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EFFECTS OF ADDITION OF MAGNESIUM, TOCOPHEROL, AND COD LIVER OIL TO A WHOLE MILK DIET ON SOME BIOCHEMICAL CONSTITUENTS IN SERUM, SOFT TISSUE, AND BONE OF CALVES[1]

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SUMMARY

Forty-eight male Guernsey calves, approximately seven days old, were fed a basal ration of whole milk plus required minerals, except magnesium, vitamin A and D, and chlortetracycline. Added per 100 lb. live weight per day to this ration, separately and in all possible combinations (2^3 factorial design), were magnesium (Mg), at 2.5 g.; D,L-α-tocopheryl acetate (T), 200 mg.; and cod liver oil (CLO), 1 oz. In anticipation of high mortality, eight calves were fed the basal ration, 12 the basal plus CLO, and eight the basal plus Mg plus CLO; this was in contrast to only four calves on the other treatments. None of the calves fed the basal ration died or exhibited signs of tetany characteristic of hypomagnesemia; however, mortality was high in the latter two groups, six and four calves, respectively. The results presented below were based upon four calves per treatment group which survived the 28-wk. experimental period. The addition of Mg resulted in a greater incidence of scours, higher terminal serum inorganic phosphorus, magnesium, and total tocopherol concentrations, lower heart and skeletal muscle calcium, greater heart and skeletal muscle magnesium (only in the absence of T from the ration), and lower calcium and higher magnesium in the bone ash. The addition of CLO produced a variety of effects: lower live weight gains and greater incidence of scours (only in the absence of T from the ration), longer P-R and Q-T electrocardiographic intervals as well as systolic indexes, greater concentrations of potassium and vitamin A in the serum, lower serum concentrations of magnesium, total tocopherol, and carotenoids, and greater whole blood urea levels. Also, the addition of CLO resulted in marked effects on many

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of the biochemical constituents determined in heart and skeletal muscle as well as liver, e.g., for muscle, lower concentrations of dry matter, ash, phosphorus, magnesium, potassium, creatine, nitrogen, and tocopherol, and greater contents of calcium and sodium. CLO, also, caused greater bone calcium and lower magnesium content. As was anticipated, the addition of T to the ration largely offset the effects of the addition of CLO. A notable exception was the absence of any effect of T on the greater electrocardiographic intervals resulting from CLO.

Prior to the study of Duncan *et al.* (15) in 1935, attempts to raise calves for an extended period of time on a whole milk diet plus a variety of supplements had generally resulted in tetany and death. These workers, stimulated by the report of Kruse *et al.* (26) on the dietary need of the rat for magnesium, were able to demonstrate normal concentrations of blood serum calcium and phosphorus but low concentrations of blood serum magnesium in calves receiving a diet of whole milk or a diet of whole milk plus various supplements containing inappreciable magnesium. Calves having low blood serum magnesium concentrations generally exhibited tetany. A subsequent investigation (22) indicated that the addition of magnesium as oxide or carbonate to a diet of whole milk, plus supplements low in magnesium content, would alleviate low serum magnesium levels as well as tetany. The principal pathological alterations observed (32) in these experiments were calcification of the yellow elastic fibers of the endocardium, aorta, jugular veins, large arteries, surfaces of the diaphragm and trabecula, and capsule of the spleen. Also, the Purkinje fibers exhibited calcification and degeneration. These findings with respect to low blood serum magnesium concentrations and tetany of calves fed a whole milk diet (plus vitamin D and trace minerals) for extended periods of time have been confirmed by several groups (25, 34, 40-42, 47, 51). However, Herman (21) was unable to produce either tetany or low blood serum magnesium concentrations in calves upon feeding a similar diet.

More recently (7, 9, 42, 45), magnesium deficiency has been studied by feeding semi-synthetic milk diets to the very young calf or by feeding milk from which magnesium was removed by an ion exchange resin (48). Possibly because of the relatively lower concentrations of magnesium in these diets in comparison with those of whole milk, magnesium deficiency developed in considerably shorter periods of time than when whole milk was fed. Although decreases in blood serum magnesium concentrations and occurrence of tetany were observed in calves fed semi-synthetic milk diets, tissue calcification was not present (9, 45).

Tocopherol deficiency in the milk-fed calf, induced by feeding cod liver oil, has resulted in a multitude of biochemical changes (6, 10, 11). In addition, cardio-vascular and skeletal muscle degeneration (muscular dystrophy) along with calcification have been observed when these tissues were subjected to histopathological examination and mineral analyses (27).

Tocopherol deficiency in the milk-fed calf not induced by feeding cod liver oil has been relatively difficult to produce; however, several groups of investigators (28, 29, 46) have recently been able to develop a dystrophogenic semi-

synthetic milk diet. The time for the appearance of muscular dystrophy using this diet was considerably longer than when cod liver oil was fed.

Similarities in the histopathological lesions of magnesium deficiency in the calf, produced by feeding for an extended period of time a whole milk diet plus various supplements low in magnesium content (32), and of tocopherol deficiency in the calf fed a milk diet consisting primarily of skimmilk plus lard and cod liver oil to induce the deficiency (27), led Blaxter *et al.* (9) to suggest that the classical histopathological changes of magnesium deficiency described in earlier work may have been the result of a concomitant tocopherol deficiency due to the inclusion of cod liver oil in the diet. However, it needs to be pointed out that in one of these studies (22) from which the histopathological material was obtained, viosterol, a source of vitamin D, was used. Recently, Thomas and Okamoto (47) have given evidence obtained visually that tocopherol will not prevent calcification of the heart and great vessels present in calves fed for extended periods of time a whole milk diet plus vitamin D and trace minerals.

The present study was undertaken to determine some of the effects from adding, separately or in all possible combinations, magnesium, tocopherol, and cod liver oil to a whole milk diet plus vitamin D, iron, manganese, copper, cobalt, and chlortetracycline, and feeding these diets for an extended period of time. This report will deal primarily with biochemical constituents; however, some statistics on growth, health, and electrocardiographic intervals will be included. A second paper (20) will report the histopathology.

EXPERIMENTAL PROCEDURE

Animals and feeding. Forty-eight one-day-old male Guernsey calves obtained from various herds in eastern Connecticut during the period June through August, 1957, were placed in sawdust-bedded individual tie-stalls in a portion of the research barn maintained at a minimum temperature of 50° F. Upon arrival, each received orally one 500-mg. oblet of chlortetracycline (Lederle's Aureomycin) and two gelatin capsules each containing 100,000 U.S.P. units of vitamin A (Nopcay 250, Type M, Lot V-9207, 250,000 U.S.P. units per gram). Twice each day, whole mixed herd milk (predominantly Holstein) was fed at the daily rate of 10% of the live weight of each calf upon arrival at the barn for the first week. If the calf had not nursed its dam, the first day's feeding consisted of colostrum from a frozen bank. Using each calf's seventh day live weight, milk was fed on the basis of 10% of live weight from eight to 14 days and thereafter on the basis of an anticipated seven-day increase of 7 lb. using the seventh day live weight as the starting value.

All calves received, from the eighth day, one of eight diets. The basal diet consisted of each calf's respective milk allowance plus, per 100 lb. of live weight per day, 140.0 mg. of iron (ferrous sulfate, 80%, exsiccated, Merck U.S.P., Lot No. 089535), 9.0 mg. of manganese (manganous sulfate, monohydrate, powder, 98%, Merck reagent, Lot No. 50946), 4.0 mg. of copper (cupric sulfate pentahydrate, fine crystals, Merck reagent, Lot No. R41478), 1.0 mg. of cobalt (cobaltous chloride hexahydrate, crystals, Baker's analyzed reagent Lot No. 7202),

2,000 U.S.P. units of preformed vitamin A (Hoffmann-LaRoche, vitamin A palmitate, gelatin beadlets, water-dispersible, containing 325,000 U.S.P. units per gram, Lot No. C-2-120-A156), 215 U.S.P. units of vitamin D_2 (Hoffmann-LaRoche, vitamin D_2, gelatin beadlets, water-dispersible, containing 35,000 U.S.P. units per gram, Lot No. C-2-120-D155), and 20.0 mg. of chlortetracycline (Lederle's Aureomycin, chlortetracycline unrefined, soluble, tinted, containing 25 g. of chlortetracycline hydrochloride per pound, Lot No. 8744-606). The minerals were supplied for each 100 lb. of live weight in 10 ml. of an aqueous solution of the following composition per liter: 47.6 g. $FeSO_4$ (80%, exsiccated), 2.8 g. $MnSO_4 \cdot H_2O$, 1.6 g. $CuSO_4 \cdot 5H_2O$, and 0.4 g. $CoCl_2 \cdot 6H_2O$ in 0.1 *N* HCl. Similarly, the vitamins were supplied in an aqueous dispersion containing 2,000 U.S.P. units of vitamin A, 215 I.U. of vitamin D_2, and 20 mg. of chlortetracycline per 10 ml. The remaining seven diets consisted of the basal plus magnesium, Mg, the basal plus tocopherol, T, the basal plus cod liver oil (cod liver oil was used to produce a tocopherol deficiency), CLO, the basal plus Mg plus T, the basal plus Mg plus CLO, the basal plus T plus CLO, or the basal plus Mg plus T plus CLO. Magnesium, as an aqueous solution containing 3.0 g. of Mg per 100 ml. ($MgCl_2 \cdot 6H_2O$, Merck crystal reagent, Lots No. D98157 and E4330), was added to provide a daily intake including the Mg in the whole milk of 2.5 g. per 100 lb. of live weight; T, as an aqueous dispersion containing 200 mg. of D,L-α-tocopherol acetate (Hoffmann-LaRoche, Rovimix E, containing 125,000 I.U. of vitamin E as D,L-α-tocopherol acetate per pound, Lot No. 091) per 10 ml., was added to provide a daily intake of 200 mg. per 100 lb. of live weight; and CLO (Nopco Chemical Co., cold pressed Icelandic cod liver oil containing 1,080 U.S.P. units of vitamin A and approximately 100 U.S.P. units of vitamin D per g., Controls No. T-9699 and 1034) was added to provide a daily intake of 1 oz. (30 ml.) per 100 lb. of live weight. The levels of magnesium, tocopherol, and cod liver oil were chosen on the basis of the statistics of Blaxter *et al.* (6-11) to provide, respectively, sufficient intake to prevent magnesium deficiency, and to prevent or to induce tocopherol deficiency. Minerals, vitamins, and cod liver oil were added, with thorough mixing, directly to the milk immediately prior to feeding. These supplements were fed on the anticipated actual live weight of each calf calculated from the current live weight and the two immediately previous seven-day increases. Stainless steel wire muzzles were used from the eighth day to the termination of the experimental period for each calf.

The first 12 calves to arrive at the research barn, the first replicate, were randomly assigned to one of the eight diets. However, in anticipation of possible mortality, two calves instead of one were assigned to the basal diet, three to the basal diet plus CLO, and two to the basal diet plus Mg plus CLO. This procedure was continued until a total of four replicates were placed on experiment. One or three days after completion of 28 successive seven-day periods on their respective diets, each calf was slaughtered.

All calves were observed three times during the day for scours and other possible disorders. Scours, if accompanied by a temperature of 103° F. or more,

were treated initially with sulfamethazine intravenously at the rate of 26.3 g. per 100 lb. live weight, followed by oral administration at the rate of 6.5 g. per 100 lb. live weight for an additional two-day period.

Average maximum and minimum barn temperatures during the experiment, with their standard errors were, respectively, 68.6 ± 0.2° F. and 58.6 ± 0.2° F. Light exposure, restricted to 12 hr. daily from incandescent sources only, averaged in intensity, at a height of 48 in. approximately in the center of each calf's stall, 4.8 ± 0.6 foot-candles.

Observations. Milk, fed and refused, was weighed to the nearest 0.1 lb. All supplements fed, i.e., minerals, vitamins, and cod liver oil, were measured to the nearest 1 ml. in glass syringes. Live weights to the nearest pound were taken every seven-day period throughout the experiment, and linear growth measurements, height at withers, heart girth, and girth of paunch, to the nearest ¼-in. on the seventh day and at the termination of the 28-wk. feeding period.

Samples of milk were obtained once weekly for the immediate determination of magnesium and calcium, the former being necessary for calculation of the amount of magnesium supplement to add to those calves' diets which included this mineral. Also, the milk samples were analyzed once every 4 wk. for tocopherol as well as carotenoids and vitamin A. Samples of cod liver oil were taken at approximately 2- to 4-wk. intervals for iodine number determinations.

Serum samples were prepared from blood obtained by jugular puncture on the seventh day and at successive 28-day periods thereafter for cholesterol, mineral, and vitamin analyses. Five days prior to slaughter, citrated whole blood was obtained similarly for terminal hemoglobin, urea, and nitrogen determinations.

Electrocardiograms were recorded as previously described (39) the week prior to subjecting each calf to its respective diet, and the 14th and 28th wk. of the feeding period.

At slaughter, the entire heart, large samples from several skeletal muscles (*biceps femoris*, *gastrocnemius*, *infraspinatus*, and *supraspinatus*) and entire liver were removed. Heart and liver were weighed. Heart and skeletal muscles were homogenized in an Omni-Mixer and the liver ground in a Hobart food chopper, sampled, and the samples held at −18° C. for subsequent mineral, creatine, cholesterol (liver), nitrogen, and vitamin analyses. The fifth left rib, left cannon bone, and tail (caudal vertebrae) bone were also removed at slaughter. The distal 30% of the length of the fifth rib, the distal 15% of the length of the cannon bone, and approximately 8 in. of the distal portion of the tail bone were held at −18° C. for mineral analyses. Other tissues were sampled for subsequent histopathological examination, and these results are to be reported in a separate paper (20).

Analyses. All analyses were in duplicate except the following in single: sodium, potassium, total tocopherol, carotenoids, and vitamin A; phosphorus in serum; creatine in heart, skeletal muscle, and liver; and ash, calcium, and magnesium in tail bone.

Magnesium and calcium contents of the whole milk were determined by titration with ethylenediamine tetraacetic acid, disodium salt (23) and averaged with their standard errors, respectively, 53.4 ± 0.7 and 501 ± 6 mg. per pound. The average tocopherol content of whole milk (37) was 0.49 ±0.05 mg. per pound, carotenoids and vitamin A contents (12) were, respectively, 21 ± 4 and 18 ± 4 γ per pound and per cent fat (4), 3.9 ± 0.1. The average iodine number of the cod liver oil as determined by the Hanus procedure (4) was 157 ± 1.

Serum calcium was determined by the procedure of Clark and Collip (18), inorganic phosphorus by the Fiske and Subbarow method (17), magnesium by precipitation as the magnesium ammonium phosphate with estimation of the latter colorimetrically by the Fiske and Subbarow method (17), serum sodium and potassium by flame photometry (3), serum total cholesterol by the method of Pearson *et al.* (35), and serum total tocopherol, carotenoids, and vitamin A as previously described (39). Whole blood was analyzed for total hemoglobin by the procedures of Evelyn and Malloy (16), urea nitrogen by the method of Folin and Svedberg as described in Hawk, Oser, and Summerson (18), and ammonia by the same procedure as urea nitrogen, except that urease was omitted.

Tissue dry matter was determined by drying in a forced-draft oven at 100 to 105° C. for 20 to 24 hr. (4). For the determination of ash, the dried sample prepared as above was placed in a muffle furnace, the temperature raised initially to 200° C., increased stepwise by 25° C. increments at one-half hour intervals to 325° C., held at 325° C. for 1 hr., increased to 520° C. and held at this temperature for approximately a 14-hr. period. Calcium in tissue was determined by flame photometry (14), after appropriate preparation of the ashed sample by the procedures of Michaels *et al.* (31). Magnesium content of tissue was determined colorimetrically (17) on the supernatant and washings from the calcium determination, essentially as for serum. Tissue was analyzed for phosphorus by the Fiske and Subbarow colorimetric procedure (17) after wet ashing, according to the procedure of Neuman as described by Peters and VanSlyke (36). Sodium and potassium content of the tissues were determined by flame photometry (3), after preparation of the samples as described by Johnston (24). The tissues were analyzed for nitrogen by the Kjeldahl procedure (4). Cholesterol content of liver was determined by the method of Sperry and Webb (44) and tissue concentration of total creatine as described by Rose *et al.* (38). Total tocopherol, and carotenoids and vitamin A (liver) were determined as previously described (39).

After removal of tissue adhering to the bone, cutting into small pieces with a band saw, removal of bone marrow under a jet of water, and partial drying in a forced-draft oven at 105° C. for 15 min., the bone was extracted initially with 250 ml. of 95% ethanol in a Soxhlet extractor for 16 hr. and, subsequently, with 250 ml. of petroleum ether (30-60° C.) for 3 hr. The lipid-free bone was ground in a laboratory Wiley mill, using a 20-mesh sieve. Approximately 1 g. of ground lipid-free bone was then ashed in a muffle furnace for 16 hr. at 525° C. Both calcium and magnesium concentrations in the ash were determined gravimetrically according to procedures of Washburn and Shear (50), as calcium

oxalate monohydrate and as magnesium ammonium phosphate hexahydrate after double precipitation of the latter.

Statistical procedures, as outlined for a 2^3 factorial design, by Cochran and Cox (13) and Snedecor (43), were used in the derivation of the statistics subsequently reported herein. Only data from calves surviving the 28-wk. supplement feeding period were used in the calculations. It was decided at the start of the experiment that if more than one calf per replicate survived this period, or if one or more calves died during the period, the lowest numbered calf in each diet-replicate grouping would be used to give four calves per diet grouping. Also, for brevity, only terminal values for serum, determined on blood samples obtained at the completion of the 28-wk. supplementation period, are reported. Neither initial values determined prior to supplementation nor average values during supplementation are included in the Results section. Average values in most cases followed the same trends as the terminal values. Also, the statistics pertaining to only two of the skeletal muscles (*biceps femoris* and *supraspinatus*) are included, since the *gastrocnemius* and *infraspinatus* exhibited essentially the same general dietary effects.

RESULTS

Feed consumption, growth, health, and electrocardiographic intervals (Table 1, Appendix Table I). Claves readily consumed their daily milk allowances with an average of 99% milk refusal free days, Appendix Table I. The addition of tocopherol to the whole milk diet resulted in a greater percentage of days free of milk refusals than those observed for calves fed no tocopherol ($+1.4 \pm 0.6$ difference using the arc sin transformation of the percentages, Table 1). The effect of tocopherol was most marked when cod liver oil was included in the diet as compared to a nonsignificant effect when cod liver oil was absent.

The daily gain in live weight during the 28-wk. supplement period averaged 1.40 lb. per calf. Tocopherol resulted in an over-all greater gain, adjusted for initial live weight, than that for the calves receiving no tocopherol. This positive growth effect of tocopherol was not consistent across all diets, for a marked response in live weight occurred in the absence of supplementary magnesium chloride versus inappreciable response in its presence. Inappreciable effect was observed in the absence of cod liver oil, versus a positive effect in its presence. The total increases in height at withers, heart girth, and girth of paunch were, with their standard deviations in parentheses, respectively, 13.5 (1.0) in., 20.6 (1.2) in., and 23.2 (2.0) in. While the increases for heart girth followed the same general dietary trends as for live weight, diet did not have a significant effect on the two other linear growth increases.

The average per cent days free of scours for the experiment was 95, Appendix Table I. The addition of magnesium chloride resulted in a marked increase in the incidence as evidenced by a decrease in the arc sin transformed percentages (Table 1) between calves fed magnesium chloride and those in which the magnesium chloride was absent. In contrast, tocopherol had no over-all effect, but

TABLE 1

Effects of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on milk refusals, growth, scours, and terminal electrocardiographic intervals of calves fed a whole milk diet

Criterion studied	Factor	Mean effect	Effect with Mg		Effect with T		Effect with CLO		Standard error: Mean effect	Standard error: Differential effect
			−[a]	+[a]	−	+	−	+		
Days free of milk refusals *(arc sin $\sqrt{\%}$)*										
	Mg	+0.4	....	....	+0.3	+0.5	−0.5	+1.3		
	T	+1.4	+1.2	+1.6	....	....	+0.5	+2.3		
	CLO	−0.4	−1.2	+0.4	−1.2	+0.4	....	....		
									0.6	0.8
Live weight increase—adjusted[b] *(lb/day)*										
	Mg	+0.02	.....	.....	+0.08	−0.04	+0.06	−0.02		
	T	+0.12	+0.18	+0.06	.....	.....	+0.04	+0.20		
	CLO	−0.02	+0.01	−0.05	−0.10	+0.06	.....	.....		
									0.03	0.04
Days free of scours *(arc sin $\sqrt{\%}$)*										
	Mg	−9.6	....	....	−5.5	−13.7	−7.6	−11.6		
	T	+0.6	+4.7	−3.5	....	.....	+4.8	− 3.6		
	CLO	−2.0	0.0	−4.0	+2.2	− 6.2	....	.....		
									1.5	2.1
Terminal electrocardiographic intervals—adjusted[c] *(sec.)*										
P − R										
	Mg	+0.004	.....	.....	+0.013	−0.005	+0.005	+0.003		
	T	+0.001	+0.010	−0.008	.....	.....	+0.005	−0.003		
	CLO	+0.021	+0.022	+0.020	+0.025	+0.017	.....	.....		
									0.007	0.010
Q − T										
	Mg	+0.002	.....	.....	+0.004	0.000	+0.005	−0.001		
	T	+0.008	+0.010	+0.006	.....	.....	−0.003	+0.019		
	CLO	+0.022	+0.025	+0.019	+0.011	+0.033	.....	.....		
									0.007	0.010
Terminal systolic index[d]										
	Mg	+0.009	.....	.....	+0.007	+0.011	+0.001	+0.017		
	T	+0.006	+0.004	+0.008	.....	.....	+0.003	+0.009		
	CLO	+0.031	+0.023	+0.039	+0.028	+0.034	.....	.....		
									0.007	0.010

[a] − indicates absence from the diet; + indicates presence.

[b] Indicates increase adjusted by covariance (13) for initial live weight observed on the seventh day prior to addition of Mg, T, or CLO to the whole milk diet.

[c] Adjusted by covariance (13) for initial values observed the week prior to addition of Mg, T, or CLO to the whole milk diet.

[d] Ratio of the Q-T interval in seconds to the square root of the time in seconds for a complete cardiac cycle.

did result in greater percentages of days free of scours in those calves receiving no supplementary magnesium or no cod liver oil. Cod liver oil also had no overall effect, but in the presence of tocopherol the incidence was greater. The number of treatments for scours averaged 0.3 per calf and was not a particular problem in this study.

None of the calves exhibited signs of tetany during the 28-wk. supplement period. Three of the four extra calves (one from each replicate) fed the basal diet were maintained on this diet for an additional 32-wk. period, with the fourth calf receiving magnesium at the level used in the experiment proper. At the

completion of this additional supplement period, the three calves fed the basal diet had average serum magnesium values of 1.0 mg. per 100 ml. (range 0.4 to 1.8), yet none exhibited tetany. The fourth calf's serum magnesium concentration was 2.3. In possible contrast, clinical signs of tocopherol deficiency, inability to arise and to drink, and subsequent death were frequent in calves fed cod liver oil without additional tocopherol. Six out of the 12 calves fed the basal diet plus cod liver oil died at average time on experiment of 22.4-wk. (range 18 to 26) and four out of the eight fed the basal diet plus cod liver oil plus magnesium at average time of 24.0-wk. (range 22 to 26). Of the calves surviving the 28-wk. supplement period and used in the statistics reported herein, one in the group fed the basal diet plus cod liver oil exhibited tocopherol deficiency signs and two in the group fed the basal diet plus cod liver oil plus magnesium.

The average heart rate in beats per minute was 99 (Appendix Table I) and this criterion was not significantly affected by diet. Cod liver oil resulted in a positive effect in the P-R and Q-T intervals (Lead II) of the electrocardiograms and also a positive effect in the systolic index. Inappreciable effect was noted on the QRS interval. These effects of cod liver oil were less pronounced upon addition of magnesium to the diet as compared to those effects in the absence of magnesium, and the results were rather inconsistent as to when tocopherol was present or absent in the diet.

Serum (Table 2 and Appendix Table II). The addition of magnesium, tocopherol, or cod liver oil resulted in inappreciable effect in the concentration of calcium in the serum determined from blood samples obtained the 28th wk. of the supplement period, Table 2. It was of interest that the average values for serum calcium during the supplement period were lower upon the addition of magnesium (-0.23 ± 0.09) and were slightly higher ($+0.16 \pm 0.09$) upon the inclusion of cod liver oil. Terminal serum phosphorus was greater when magnesium was added to the diet (Table 2).

Added magnesium, Table 2, resulted in appreciably higher terminal serum concentrations of this element, the effect of tocopherol was also positive, but cod liver oil had the opposite effect. The response to magnesium varied some in magnitude in the presence or absence of either tocopherol or cod liver oil, but in all cases it was positive and statistically significant. In possible contrast, cod liver oil resulted in markedly lower serum magnesium concentrations in the absence of magnesium in the diet, but insignificant effect in its presence. Tocopherol resulted in a positive effect on serum magnesium concentrations in the absence of added dietary magnesium and little response in its presence. In the absence of cod liver oil, tocopherol had an insignificant effect on serum magnesium concentration; but in the presence of cod liver oil, tocopherol resulted in a positive response.

Terminal serum sodium values varied inappreciably from diet to diet. In contrast, terminal serum potassium concentrations were lower upon the addition of tocopherol to the diet and the opposite was found for cod liver oil. The effect of tocopherol was not consistent across all diets, e.g., in the absence of magnesium,

TABLE 2

Effect of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on some biochemical constituents in serum and on whole blood urea of calves fed a whole milk diet [a]

Criterion studied	Factor	Mean effect	Effect with Mg		Effect with T		Effect with CLO		Standard error: Mean effect	Standard error: Differential effect
			−[b]	+[b]	−	+	−	+		
		(mg/100 ml.)								
Calcium										
	Mg	−0.15	.....	.....	+0.17	−0.48	+0.07	−0.37		
	T	+0.10	+0.42	−0.22	.....	.....	+0.08	+0.12		
	CLO	+0.14	+0.35	−0.07	+0.12	+0.16	.....	.....		
									0.18	0.26
Inorganic phosphorus										
	Mg	+1.3	....	....	+1.3	+1.3	+0.9	+1.7		
	T	+0.2	+0.2	+0.2	....	....	−0.2	+0.6		
	CLO	+0.2	−0.2	+0.6	−0.2	+0.6	....	....		
									0.2	0.3
Magnesium										
	Mg	+0.83	.....	.....	+1.01	+0.65	+0.48	+1.18		
	T	+0.22	+0.40	+0.04	.....	.....	+0.15	+0.29		
	CLO	−0.50	−0.85	−0.15	−0.57	−0.43	.....	.....		
									0.10	0.14
Potassium										
	Mg	+0.4	....	....	−0.1	+0.9	−0.1	+0.9		
	T	−0.9	−1.5	−0.3	....	....	−0.2	−1.6		
	CLO	+1.1	+0.6	+1.6	+1.8	+0.4	....	....		
									0.4	0.5
Cholesterol										
	Mg	+26	....	....	+18	+34	+32	+20		
	T	+13	+ 5	+21	....	....	−13	+39		
	CLO	−26	−20	−32	−52	0	....	....		
									14	20
		(γ/100 ml.)								
Tocopherol										
	Mg	+ 94	.....	.....	− 10	+198	+122	+ 66		
	T	+653	+549	+757	.....	.....	+651	+655		
	CLO	−124	− 95	−153	−126	−122	.....	.....		
									46	65
		(mg. N°/100 ml.)								
Whole blood urea										
	Mg	+0.4	....	....	+1.0	−0.2	+0.6	+0.2		
	T	−1.3	−0.7	−1.9	....	....	−0.8	−1.8		
	CLO	+0.9	+1.1	+0.7	+1.4	+0.4	....	....		
									0.4	0.6

[a] Represent values obtained the 28th wk. of the supplement period.
[b] − indicates absence from the diet; + indicates presence.
[c] Adjusted by covariance (13) for initial values obtained the day prior to the addition of supplements to the diet.

a negative response in serum potassium was observed, but in its presence no significant effect was found. Also, in the absence of cod liver oil, tocopherol had little effect on serum potassium, but in its presence, a marked negative effect. The addition of cod liver oil to the whole milk diet resulted in an appreciable response in the serum potassium concentration only in the presence of magnesium in the diet or in the absence of tocopherol.

Terminal serum cholesterol tended to be greater when magnesium was included in the diet and lower upon the addition of cod liver oil. The effect of magnesium was greater in the presence of tocopherol and in the absence of cod

liver oil in the diets. The over-all negative effect of cod liver oil was due to a marked response in the absence of tocopherol in the diet with the remaining differential responses, i.e., in the presence or absence of magnesium or presence of tocopherol in the diets, being nonsignificant. While tocopherol did not exhibit an over-all effect on serum cholesterol, it did tend to result in greater concentrations of this constituent when cod liver oil was included in the diet.

Serum tocopherol concentrations were slightly greater upon inclusion of magnesium in the diet, markedly greater with tocopherol, and lower with cod liver oil. The effects of magnesium on the concentrations of this vitamin in the serum were most pronounced in the presence of tocopherol in the diet and in the absence of cod liver oil. The positive effects of tocopherol were quite consistent whether magnesium or cod liver oil was absent or present in the diet. The depressing effect of cod liver oil was most pronounced in the presence of magnesium. Terminal concentrations of serum carotenoids were found to be lower (-30 ± 4) and serum vitamin A greater ($+8 \pm 4$) upon inclusion of cod liver oil in the diet.

Whole blood hemoglobin and ammonia determined in samples obtained just prior to the termination of the experiment averaged, with their standard deviations in parentheses, 11.6 (1.2) g. per milliliter and 4.4 (1.0) mg. NH_3 per 100 milliliters, respectively. Neither criterion was appreciably affected by diet. Whole blood urea was lower in those calves receiving tocopherol in the diet and higher with cod liver oil. The negative effect of tocopherol was significant when magnesium was present in the diet and also when cod liver oil was present. Cod liver oil's positive effect on whole blood urea was significant when either magnesium or tocopherol was absent from the diet.

Heart and skeletal muscle (Tables 3 and 4 and Appendix Table III.)[6] The average weight of the heart was 892 g., with a standard deviation of 106. Cod liver oil when added to the whole milk diet resulted in a positive response ($+168 \pm 38$ g.). This effect of cod liver oil was somewhat consistent in the absence or presence of magnesium ($+179 \pm 53$ and 157 ± 53, respectively), but in the absence of tocopherol, the effect ($+206 \pm 53$) of cod liver oil was of greater magnitude than in its presence ($+130 \pm 53$).

The dry matter contents of the heart and skeletal muscle tissue were generally less when cod liver oil was added to the diet than in its absence (heart, -0.2 ± 0.2 g. of wet tissue, *biceps femoris*, -0.8 ± 0.2, and *supraspinatus*, -0.6 ± 0.2). Tocopherol had the opposite effect (heart, $+1.0 \pm 0.2$, *biceps femoris*, $+0.6 \pm 0.2$, and *supraspinatus*, $+0.3 \pm 0.2$). These over-all effects of cod liver oil or of tocopherol were not influenced by the presence or absence of magnesium in the diet. Cod liver oil's depressing effect on dry matter content was counteracted by tocopherol, as evidenced by positive changes in the dry matter when tocopherol was included (e.g., heart, -1.5 ± 0.2 in the absence of tocopherol

[6] The concentration of all biochemical constituents was calculated on the basis of per 100 g. of dry matter as well as the concentration of the minerals on the basis of per 100 g. of ash. However, since the statistics calculated on these two bases were essentially similar as to dietary effects to those on a wet basis, they are not presented here.

TABLE 3

Effect of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on calcium, phosphorus, and magnesium in heart and skeletal muscle of calves fed a whole milk diet [a]

Criterion studied	Factor	Mean effect	Effect with Mg		Effect with T		Effect with CLO		Standard error: Mean effect	Standard error: Differential effect
			−[b]	+[b]	−	+	−	+		
		(mg/100 g. wet tissue)								
Calcium										
Heart										
	Mg	−0.7	....	....	−1.6	+0.2	0.0	−1.4		
	T	−2.0	−2.8	−1.2	....	....	+0.3	−4.3		
	CLO	+2.0	+2.7	+1.3	+4.3	−0.3	....	....		
									0.4	0.6
Biceps femoris										
	Mg	−0.2	....	....	−0.2	−0.2	−0.2	−0.2		
	T	−0.9	−0.9	−0.9	....	....	−0.1	−1.7		
	CLO	+0.9	+0.9	+0.9	+1.7	+0.1	....	....		
									0.1	0.1
Supraspinatus										
	Mg	−0.4	....	....	−0.4	−0.4	−0.4	−0.4		
	T	−1.2	−1.2	−1.2	....	....	−0.1	−2.3		
	CLO	+1.0	+1.0	+1.0	+2.1	−0.1	....	....		
									0.1	0.2
Phosphorus										
Heart										
	Mg	+ 1	....	....	+ 1	+1	+2	0		
	T	+13	+13	+13	....	....	−7	+33		
	CLO	−13	−12	−14	−33	+7	....	....		
									2	2
Biceps femoris										
	Mg	+ 2	....	....	+ 2	+2	+1	+ 3		
	T	+17	+17	+17	....	....	+1	+33		
	CLO	−14	−15	−13	−30	+2	....	....		
									2	3
Supraspinatus										
	Mg	+3	....	....	+ 6	0	+1	+ 5		
	T	+7	+10	+4	....	....	−3	+17		
	CLO	−8	−10	−6	−18	+2	....	....		
									2	3
Magnesium										
Heart										
	Mg	+0.4	....	....	+0.9	−0.1	+0.6	+0.2		
	T	+2.0	+2.5	+1.5	....	....	−1.0	+5.0		
	CLO	−2.6	−2.4	−2.8	−5.6	+0.4	....	....		
									0.3	0.4
Biceps femoris										
	Mg	+0.2	....	....	+0.2	+0.2	0.0	+0.4		
	T	+2.6	+2.6	+2.6	....	....	−0.5	+5.7		
	CLO	−3.1	−3.3	−2.9	−6.2	0.0	....	....		
									0.3	0.4
Supraspinatus										
	Mg	+0.6	....	....	+1.3	−0.1	+0.2	+1.0		
	T	+1.5	+2.2	+0.8	....	....	−1.0	+4.0		
	CLO	−1.6	−2.0	−1.2	−4.1	+0.9	....	....		
									0.4	0.6

[a] Represent values obtained upon completion of the 28-wk. supplement period.
[b] − indicates absence from the diet; + indicates presence.

TABLE 4

Effect of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on sodium, potassium, and tocopheral concentrations in heart and skeletal muscle of calves fed a whole milk diet [a]

Criterion studied	Factor	Mean effect	Effect with Mg		Effect with T		Effect with CLO		Standard error: Mean effect	Standard error: Differential effect
			−[b]	+[b]	−	+	−	+		
		(mg/100 g. wet tissue)								
Sodium										
Heart										
	Mg	− 2	....	....	− 2	− 2	− 2	− 2		
	T	−20	−20	−20	....	....	+ 4	−44		
	CLO	+15	+15	+15	+40	−10	....	....		
									2	2
Biceps femoris										
	Mg	0	....	....	0	0	0	0		
	T	−24	−24	−24	....	....	0	−48		
	CLO	+25	+24	+26	+50	0	....	....		
									3	4
Supraspinatus										
	Mg	− 1	....	....	− 2	0	− 1	− 1		
	T	−15	−16	−14	....	....	0	−30		
	CLO	+15	+15	+15	+30	0	....	....		
									2	2
Potassium										
Heart										
	Mg	+ 6	....	....	− 2	+14	+ 6	+ 6		
	T	+16	+ 7	+25	....	....	−13	+45		
	CLO	−13	−13	−13	−42	+16	....	....		
									7	10
Biceps femoris										
	Mg	− 2	....	....	−14	+10	0	− 4		
	T	+34	+21	+47	....	....	− 7	+75		
	CLO	−46	−44	−48	−87	− 5	....	....		
									12	16
Supraspinatus										
	Mg	− 1	....	....	+ 8	−10	− 2	0		
	T	+22	+31	+13	....	....	−12	+56		
	CLO	−26	−27	−25	−60	+ 8	....	....		
									7	10
		(log γ/100 g. wet tissue)								
Tocopherol										
Heart										
	Mg	+0.04	.....	.....	+0.07	+0.01	+0.06	+0.02		
	T	+0.70	+0.73	+0.67	.....	.....	+0.65	+0.75		
	CLO	−0.09	−0.07	−0.11	−0.14	−0.04	.....	.....		
									0.05	0.07
Supraspinatus										
	Mg	−0.01	.....	.....	−0.03	+0.01	−0.02	0.00		
	T	+0.71	+0.69	+0.73	.....	.....	+0.63	+0.79		
	CLO	−0.13	−0.14	−0.12	−0.21	−0.05	.....	.....		
									0.02	0.03

[a] Represent values obtained upon completion of the 28-wk. supplement period.
[b] − indicates absence from the diet; + indicates presence.

and $+1.1 \pm 0.2$ in the presence of tocopherol). Conversely, tocopherol had little effect in the absence of cod liver oil (e.g., heart, -0.3 ± 0.2), but a marked effect in its presence (e.g., heart, $+2.3 \pm 0.2$). Essentially similar dietary trends as those for dry matter were found for ash content.

Magnesium added to the whole milk diet resulted in lower calcium concentration of the tissues (Table 3). Tocopherol also had a negative effect while, in contrast, cod liver oil gave positive responses. The effects of added magnesium on calcium concentration were reasonably consistent in the absence or presence of either tocopherol or cod liver oil for the two skeletal muscles. However, for heart, magnesium resulted in negative responses in the absence of tocopherol and in the presence of cod liver oil, but resulted in inappreciable effect either in the presence of tocopherol or in the absence of cod liver oil. The negative effect of tocopherol on calcium content of the tissues was essentially similar whether or not magnesium was present. However, in the absence of cod liver oil, tocopherol had little effect as contrasted to the marked negative effects of tocopherol in the presence of cod liver oil. Conversely, the positive effects of cod liver oil on calcium content were inappreciable in the presence of tocopherol but marked in its absence.

The phosphorus contents of heart and skeletal muscle (Table 3) were greater in calves fed tocopherol and lower in those fed cod liver oil. Magnesium was without significant effect on this element. The positive response of tocopherol on phosphorus content was marked in the presence of cod liver oil and either small or insignificant in the absence of cod liver oil. Conversely, the negative effect of cod liver oil was evident only in those calves not receiving tocopherol, since, in the presence of tocopherol, cod liver oil resulted in small, nonsignificant decreases or increases in phosphorus.

The effects of the addition to the whole milk diet of both tocopherol and cod liver oil on the magnesium contents of heart and skeletal muscle (Table 3) were essentially similar in trend to those for phosphorus.

The sodium contents of these tissues (Table 4) were unaffected by the addition of magnesium to the diet, but negative responses were observed due to the addition of tocopherol and positive responses due to the addition of cod liver oil. The converse was found for potassium: tocopherol resulted in a positive effect, whereas negative effects were observed for cod liver oil. Also, the ratio of potassium to sodium in these tissues (which on a wet tissue basis averaged 3.66 for heart, 5.94 for *biceps femoris,* and 5.44 for *supraspinatus,* with standard deviations of 0.34, 0.66, and 0.45, respectively) were greater when tocopherol was present in the diet ($+0.80 \pm 0.12$ for heart, $+1.77 \pm 0.23$ for *biceps femoris,* and $+1.22 \pm 0.16$ for *supraspinatus*) and smaller upon the addition of cod liver oil (-0.51 ± 0.12 for heart, -1.99 ± 0.23 for *biceps femoris,* and -1.26 ± 0.16 for *supraspinatus*). The effects of tocopherol on both sodium and potassium and their ratios were marked in the presence of cod liver oil and the effects of cod liver oil marked in the absence of tocopherol.

Creatine and nitrogen (Appendix Table III) concentrations of heart and muscle tissue were greater in the presence of tocopherol in the diet and in the absence of cod liver oil. The mean responses for tocopherol were, respectively, $+0.09 \pm 0.02$ g. nitrogen per 100 g. wet tissue and $+46 \pm 5$ mg. creatine per 100 g. of wet tissue for heart, $+0.12 \pm 0.04$ and $+75 \pm 8$ for *biceps femoris,* and $+0.06 \pm 0.03$ and $+47 \pm 6$ for *supraspinatus.* Those for cod liver oil were, re-

spectively, -0.04 ± 0.02 and -37 ± 5 for heart, -0.15 ± 0.04 and -50 ± 8 for *biceps femoris*, and -0.07 ± 0.03 and -33 ± 6 for *supraspinatus*. The effects of tocopherol were attributable primarily to the inclusion of cod liver oil in the diet and those for cod liver oil to the absence of tocopherol.

The lipid content (Appendix Table III) of heart was greater ($+0.32 \pm 0.11$ g. per 100 g. of wet tissue) in those calves fed tocopherol, but not significantly affected by cod liver oil (-0.17 ± 0.11) or magnesium (-0.01 ± 0.11). *Supraspinatus* lipids were lower in the presence of tocopherol (-0.22 ± 0.10) and greater in the presence of cod liver oil ($+0.37 \pm 0.10$). While the effects of tocopherol on heart lipids in the presence of cod liver oil and those of cod liver oil in absence of tocopherol tended to follow the general pattern as cited above for some of the previous constituents, such as phosphorus, magnesium, sodium, etc., these were not as pronounced in the case of the lipids. No such trends were observable for *supraspinatus*.

The concentration of tocopherol in heart and *supraspinatus* (Table 4) were markedly greater when tocopherol was fed and slightly lower upon inclusion of cod liver oil in the diet. The effect of tocopherol was of approximately the same magnitude whether magnesium or cod liver oil was or was not included in the diet. The effect of cod liver oil was not consistent across all diets, since negative responses occurred in the absence of tocopherol, in contrast to nonsignificant effects in the presence of tocopherol.

Liver (Table 5 and Appendix Table IV). Tocopherol resulted in smaller liver weights (Appendix Table IV), as evidenced by an over-all negative response (-206 ± 111 g.) to the addition of this vitamin to the whole milk diet. The negative effect of tocopherol was especially apparent upon the addition of either magnesium (-332 ± 156) or cod liver oil (-470 ± 156) to the diet. Conversely, calves fed cod liver oil had larger livers ($+482 \pm 111$) than those not fed this substance, and the effect of cod liver oil was quite pronounced ($+746 \pm 156$) in the absence of tocopherol. Similar dietary trends were observed for the effect of tocopherol on dry matter content ($+0.6 \pm 0.3$ g. per 100 g. wet tissue for the over-all effect, $+0.8 \pm 0.4$ in the presence of magnesium, and $+0.8 \pm 0.4$ in the presence of cod liver oil); however, no significant responses were observed for cod liver oil.

Liver creatine concentrations were less in calves fed cod liver oil, with the negative effect of cod liver oil being more evident when either magnesium or tocopherol was absent from the diet. Liver cholesterol concentrations were also less when cod liver oil was added to the diet, with this negative effect being significant only in the presence of magnesium and in the absence of tocopherol. The concentration of lipids in the liver of calves fed cod liver oil was greater than that in livers of calves not fed this substance. This positive effect of cod liver oil was not counteracted by tocopherol for, in the presence of tocopherol, the effect was significantly larger and in the absence of tocopherol, inappreciable. The over-all effect of tocopherol on lipid concentration was not significant; however, the effect in the presence of cod liver oil was positive.

Liver tocopherol concentrations (Table 5) were inappreciably affected by

TABLE 5

Effects of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on some biochemical constituents in liver of calves fed a whole milk diet [a]

Criterion studied	Factor	Mean effect	Effect with Mg		Effect with T		Effect with CLO		Standard error: Mean effect	Standard error: Differential effect
			−[b]	+[b]	−	+	−	+		
		(mg/100 g. wet tissue)								
Creatine										
	Mg	− 2	....	....	− 3	− 1	− 4	0		
	T	− 3	− 4	− 2	....	....	− 5	− 1		
	CLO	− 6	− 8	− 4	− 8	− 4	....	....		
									2	4
Cholesterol										
	Mg	+ 3	....	....	+ 9	− 3	+17	−11		
	T	−10	− 4	−16	....	....	−31	+11		
	CLO	−27	−13	−41	−48	− 6	....	....		
									12	16
		(g/100 g. wet tissue)								
Lipids										
	Mg	−0.02	.....	.....	−0.07	+0.03	+0.03	−0.07		
	T	+0.11	+0.06	+0.16	.....	.....	+0.01	+0.21		
	CLO	+0.21	+0.26	+0.16	+0.10	+0.32	.....	.....		
									0.07	0.10
		(log γ/100 g. wet tissue)								
Tocopherol										
	Mg	−0.01	.....	.....	−0.01	−0.01	−0.02	0.00		
	T	+0.96	+0.96	+0.96	.....	.....	+0.92	+1.00		
	CLO	−0.18	−0.19	−0.17	−0.22	−0.14	.....	.....		
									0.04	0.05

[a] Represent values obtained upon completion of the 28-wk. supplement period.
[b] − indicates absence from the diet; + indicates presence.

the inclusion of magnesium in the diet. The effect of tocopherol was positive, and that for cod liver oil, negative. While the effect of magnesium on the concentration of carotenoids was inappreciable, tocopherol resulted in a positive response ($+0.31 \pm 0.07$) and cod liver oil, negative (-0.46 ± 0.07). In the presence of cod liver oil in the diet, the effect of tocopherol was considerable ($+0.70 \pm 0.10$); whereas, in the absence of cod liver oil, the effect was negligible (-0.08 ± 0.10). Similarly, cod liver oil resulted in a marked negative response (-0.85 ± 0.10) in the absence of tocopherol in the diet, but in the presence of tocopherol, an inappreciable effect (-0.07 ± 0.10) was observed. The responses of liver vitamin A to tocopherol and cod liver oil were positive ($+0.39 \pm 0.04$ and $+0.92 \pm 0.04$, respectively). The effect of tocopherol in the absence of cod liver oil was less ($+0.15 \pm 0.06$) than in the presence of this substance ($+0.63 \pm 0.06$). A similar difference in magnitude of the effect of cod liver oil was observed in the absence ($+0.68 \pm 0.06$) and in the presence ($+1.16 \pm 0.06$) of added tocopherol.

Bone (Table 6 and Appendix Table V). Ash contents of rib, cannon, and tail bones were, respectively, 57.5, 58.3, and 54.7 g. per 100 g. of lipid-free bone, with standard deviations of 1.3, 1.9, and 1.7. Ash content was not appreciably affected by diet.

TABLE 6

Effects of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on calcium and magnesium concentrations per unit of ash in bone of calves fed a whole milk diet[a]

Criterion studied	Factor	Mean effect	Effect with Mg		Effect with T		Effect with CLO		Standard error: Mean effect	Standard error: Differential effect
			−[b]	+[b]	−	+	−	+		
		(g/100 g. ash)[c]								
Calcium										
Rib										
	Mg	−0.28	.....	.....	−0.33	−0.23	−0.22	−0.34		
	T	−0.07	−0.11	−0.03	.....	.....	−0.09	−0.05		
	CLO	+0.17	+0.23	+0.11	+0.14	+0.20	.....	.....		
									0.08	0.11
Cannon										
	Mg	−0.27	.....	.....	−0.40	−0.14	−0.10	−0.44		
	T	−0.19	−0.32	−0.06	.....	.....	−0.13	−0.25		
	CLO	+0.09	+0.26	−0.08	+0.16	+0.02	.....	.....		
									0.09	0.12
Tail										
	Mg	−0.44	.....	.....	−0.52	−0.36	−0.36	−0.52		
	T	−0.08	−0.15	−0.01	.....	.....	+0.01	−0.17		
	CLO	+0.11	+0.19	+0.03	+0.20	+0.02	.....	.....		
									0.04	0.06
Magnesium										
Rib										
	Mg	+0.21	.....	.....	+0.23	+0.19	+0.11	+0.31		
	T	+0.05	+0.07	+0.03	.....	.....	+0.04	+0.06		
	CLO	−0.06	−0.16	+0.04	−0.08	−0.04	.....	.....		
									0.01	0.04
Cannon										
	Mg	+0.20	.....	.....	+0.25	+0.15	+0.10	+0.30		
	T	+0.06	+0.10	+0.02	.....	.....	+0.10	+0.02		
	CLO	−0.08	−0.18	+0.02	−0.12	−0.04	.....	.....		
									0.03	0.04
Tail										
	Mg	+0.21	.....	.....	+0.25	+0.17	+0.12	+0.30		
	T	+0.06	+0.10	+0.02	.....	.....	+0.03	+0.09		
	CLO	−0.07	−0.16	+0.02	−0.10	−0.04	.....	.....		
									0.02	0.03

[a] Represent values obtained upon completion of the 28-wk. supplement period.
[b] − indicates absence from the diet; + indicates presence.
[c] Lipid-free basis after extraction with ethyl alcohol and petroleum ether.

Magnesium added to the diet resulted in negative responses in bone calcium. Upon inclusion of tocopherol in the diet, the over-all effects were negative but not of the same magnitude as for those effects of added magnesium. Cod liver oil tended to cause slight positive responses. The negative responses to magnesium were generally less in the presence of tocopherol in the diet and in the absence of cod liver oil than in the absence of tocopherol and in the presence of cod liver oil. The responses for tocopherol were generally significantly negative in the absence of magnesium and in the presence of cod liver oil. Conversely, the positive effect of cod liver oil was most pronounced in the absence of magnesium and tocopherol.

Magnesium added to the whole milk diet resulted in greater bone magnesium concentrations, as evidenced by the over-all positive responses to this element.

Tocopherol caused positive responses, but these were of considerably smaller magnitude than the effects attributable to the addition of magnesium. Cod liver oil had a negative effect on bone magnesium content. The positive effects of magnesium were generally less in the presence of tocopherol and in the absence of cod liver oil than in the opposite cases. The positive effects of tocopherol on bone magnesium concentrations were of considerably smaller magnitude when magnesium was added to the diet and when cod liver oil was absent, than when the magnesium was absent or cod liver oil present. The negative effects of cod liver oil were especially noticeable in the absence of magnesium and tocopherol from the diets.

The ratio of calcium concentration to magnesium concentration in bone averaged 52 for rib, 56 for cannon, and 59 for tail, with their respective standard deviations being 11, 15, and 12. The addition of magnesium caused smaller ratios, as evidenced by negative responses (-17 ± 4 for rib, -21 ± 5 for cannon, and -24 ± 4 for tail) upon addition of this element to the whole milk diet. Tocopherol also resulted in negative effects (-7 ± 4, -11 ± 5, and -10 ± 4), whereas the effect of cod liver oil was positive ($+8 \pm 4$, $+12 \pm 5$, and $+12 \pm 4$). The effects of the addition of magnesium were most evident in the absence of tocopherol from the diet and in the presence of cod liver oil; those of tocopherol, in the absence of magnesium and in the presence of cod liver oil; and those of cod liver oil, in the absence of either magnesium or tocopherol.

DISCUSSION

The data confirm by biochemical means the previous observations (32, 47), of greater calcium concentration in soft tissue in the calf fed for extended periods of time a diet consisting of whole milk plus vitamins A and D, chlortetracycline, and minerals other than magnesium, than when the same diet is supplemented with magnesium. Similar calcification occurring in the guinea pig and rat has been reported (30, 33) when diets low in magnesium content were fed. While the addition of magnesium to these diets prevented the increases in the calcium content of soft tissue, whether the calcification was due to hypomagnesemia per se or indirectly to an interrelationship(s) between magnesium and another mineral(s) or other dietary constituent(s) is not known. Some evidence for possible interrelationships between diet content of either magnesium and calcium or magnesium and phosphorus on soft tissue calcification has been obtained for the guinea pig by Maynard *et al.* (30). More recently, O'Dell *et al.* (33) have indicated that in both guinea pigs and rats, excessive dietary phosphorus to magnesium ratios are more conducive to calcification of soft tissue than excessive calcium to magnesium ratios. Tocopherol in the present experiment did not appear to influence the negative responses in calcium content of the tissues due to the presence of magnesium in the diet. Evidence for this was that the negative effects of additional magnesium on tissue calcification were, in the case of heart, greater in the absence of tocopherol in the diet than in its presence. In the case of skeletal muscle, inappreciable differences occurred in the responses whether tocopherol was absent or present in the diet. It would,

therefore, appear that tocopherol per se had little effect in alleviating elevated calcium concentration of soft tissue due to low magnesium content of the diet, as suggested by Blaxter and Rook (9).

In contrast to the occurrence of soft tissue calcification in chronic hypomagnesemia of calves, acute hypomagnesemia, produced in a relatively shorter period of time by feeding semi-synthetic milk diets containing less magnesium than whole milk, has not resulted in calcification as determined by visual inspection (45) or histopathological study (9). However, it is of interest that in one of these studies (9), the average calcium contents of eight out of 11 tissues of three magnesium-deficient calves were greater than the averages of the same tissues of four normal calves. While the statistical significance of these data remain doubtful, they would tend to support the biochemically determined increased calcium content of soft tissue as reported herein and of those for the guinea pig (30).

The absence in this experiment of any visual signs in the intact calf characteristic of hypomagnesemia, such as those carefully described by Blaxter and Rook (9), is contrary to most of the reports in the literature as reviewed in the introduction. Comparison of the serum and especially bone magnesium concentrations of calves of this study not fed magnesium with those in the literature of calves exhibiting tetany (9, 34, 42, 47), indicated that the degree of magnesium deficiency was considerably less in the present experiment. The reasons for the milder hypomagnesemia in the calves of this experiment are not known. Possibly, the relatively slower rates of gain in live weight as observed in this experiment as contrasted to those of some of the other reported experiments (9, 40, 47), may have contributed to a less rapid magnesium depletion. In contradiction of this, Parr (34) has given evidence to indicate little difference in depletion rate between calves fed whole milk at the rate of 10% of live weight and those fed at the 20% level. Another possible causative factor might have been the lack of exercise, since the calves were maintained in tie-stalls except for a short walk weekly to be weighed. Also, the temperature of the unit in which the calves were housed was maintained at a minimum of 50° F. Had the temperature been allowed to fall below this level, it might have resulted in a more rapid magnesium depletion, because low ambient temperature has been reported to increase considerably the magnesium required per gram of diet to promote equal gain in weight of rats (19). Also, there may exist a breed difference (45), since previous investigators have used breeds other than Guernseys to study hypomagnesemia.

Most of the other observations pertaining to hypomagnesemia are essentially in agreement with the previous reports in the literature. For example, both serum and bone magnesium concentrations were markedly greater in calves which had this element added to the whole milk diet (9, 34, 40, 47). In contrast, the presence of magnesium in the diet had little effect upon magnesium concentration of soft tissue (9, 34), with the exception that, in the absence of tocopherol in the diet, magnesium content of soft tissue was greater than in its presence. Both serum (average values for the supplement period but not terminal values)

and bone calcium concentrations were greater in calves not fed magnesium than were those in calves fed magnesium (9, 34). Cholesterol values tended to be lower in calves fed the whole milk diet than in those calves fed whole milk plus magnesium (49). Possible exceptions were lower serum inorganic phosphorus and tocopherol concentrations occurring in the calves fed no added magnesium as compared to those occurring in calves fed additional magnesium. Scours were not a particular problem in calves not fed additional magnesium as magnesium chloride, but the addition of this compound caused markedly greater incidence. The effect of this higher incidence of scouring on the calf was not ascertained, since balance or digestibility studies were not undertaken.

The rather marked effects of cod liver oil on the biochemical constituents of serum and soft tissue, and the almost opposite effects of tocopherol, are essentially in agreement with the excellent and detailed studies of Blaxter *et al.* (6, 9-11). Possible exceptions were the negative responses to cod liver oil of ash content of wet tissue (or on a dry matter basis) and on phosphorus and magnesium contents of wet tissue, as well as the negative effects of cod liver oil on tocopherol content of serum and soft tissue and the positive effects of tocopherol on the tocopherol concentrations of these same tissues. Differences between the experimental procedures and animals might explain in part these apparent discrepancies. For example, the present study dealt with chronic tocopherol deficiency produced by cod liver oil added to a whole milk diet containing tocopherol; whereas, in the studies of Blaxter *et al.* an acute tocopherol deficiency was probably produced due to the use of a semi-synthetic milk containing inappreciable tocopherol. Also, in the present study, data described herein refer only to calves surviving the 28-wk. supplement period; whereas, the Scottish experiments comprised data from all calves, including those which died from cod liver oil toxicity. Thus, in the study reported herein, a selected group of calves possibly resistant to cod liver oil toxicity were used as contrasted to all calves in the latter experiments. The lack of response of tissue tocopherol concentration to tocopherol supplementation or the absence of a negative effect of cod liver oil on tissue tocopherol content observed by Blaxter *et al.*, as contrasted to the responses observed herein may have, in part, been due to differences in analytical procedures, e.g., molecular distillation used in the procedure for tocopherol in this study but not in those reported by Blaxter *et al.*

Slight heart dysfunction resulted from cod liver oil, as evidenced by longer electrocardiographic intervals, P-R and Q-T, as well as by an increase in the systolic index. Since tocopherol did not have an appreciable effect either on the intervals or systolic index, it would appear possible, as recently discussed by Adams *et al.* (1), that tocopherol per se may not be involved in the heart malfunction. These increases in the electrocardiographic intervals due to cod liver oil confirm the earlier observations of Agduhr and Stenström (2) but not those of Barnes *et al.* (5).

Cod liver oil accentuated the hypomagnesemia produced by the whole milk diet, since it caused negative responses in the concentrations of magnesium in

serum, heart and muscle tissue, and bone. These effects of cod liver oil were partially counteracted by positive responses due to the addition of tocopherol to the diet or to the addition of magnesium. In the case of serum and bone, the effects of additional magnesium were of considerably greater magnitude than the effects of tocopherol. While the possible explanation (29) of this lowered magnesium concentration of tissues due to cod liver oil is the formation of soaps owing to the combination of free fatty acids (formed from the hydrolysis of the glycerides of cod liver oil), this undoubtedly does not account for the activity of tocopherol in reducing the magnesium depletion of the tissues. Also, it does not explain either the marked increase in calcium content of the soft tissues due to addition of cod liver oil to the diet or the slight increases in calcium content of bone. To definitely answer these points, mineral balance studies are undoubtedly needed; however, the results from this study would appear to indicate that basic relationships other than the formation of soaps underlie the effects of cod liver oil in depleting the body reserves of magnesium and its partial or complete rectification by adding either tocopherol or magnesium to the whole milk diet.

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APPENDIX TABLE I

Effects of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on milk refusals, growth, scours, and terminal electrocardiographic intervals (Lead II) of calves fed a whole milk diet [a]

	Treatments [b]								
Mg	−	+	−	−	+	+	−	+	Standard
T	−	−	+	−	+	−	+	+	deviation
Criterion CLO	−	−	−	+	−	+	+	+	per calf
Days free of milk refusals									
%	100.0	99.8	99.9	99.5	100.0	99.9	100.0	100.0	....
Arc sin $\sqrt{\%}$	90.0	88.0	89.0	86.5	90.0	89.0	90.0	90.0	1.6
Live weight [c] *(lb/day)*									
Increase	1.41	1.37	1.38	1.19	1.43	1.35	1.64	1.44	0.12
Adjusted	1.36	1.42	1.41	1.24	1.46	1.34	1.56	1.43	0.09
Days free of scours									
%	98.0	92.5	99.1	97.4	96.4	95.9	99.4	84.8	....
Arc sin $\sqrt{\%}$	82.1	74.9	87.3	82.5	79.3	78.8	86.8	67.4	4.3
Heart rates [d] *(beats/min)*	100	98	109	108	94	100	86	102	11
Terminal electrocardiographic intervals [d] *(sec.)*									
P-R	0.14	0.14	0.14	0.15	0.15	0.18	0.17	0.15	0.02
QRS	0.07	0.06	0.07	0.07	0.06	0.08	0.09	0.06	0.01
QT	0.30	0.29	0.28	0.30	0.30	0.31	0.33	0.32	0.02
Terminal systolic index [e]	0.371	0.374	0.375	0.395	0.375	0.406	0.398	0.420	0.021

[a] Values represent four calves per diet group.

[b] − indicates absence from the diet; + indicates presence.

[c] Increase from the seventh to 203rd day (28th-wk.) on experiment. Adjusted indicates increase adjusted by covariance (13) for initial live weight observed on the seventh day prior to addition of Mg, T, or CLO to the whole milk diet.

[d] All values were obtained the 28th wk. of the supplement period adjusted by covariance (13) for initial values observed the week prior to addition of Mg, T, or CLO to the whole milk diet.

[e] The ratio of the QT interval to the square root of the time in seconds for a complete cardiac cycle, unadjusted values.

APPENDIX TABLE II

Effects of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on terminal calcium, inorganic phosphorus, magnesium, sodium, potassium, cholesterol, tocopherol, carotenoids, vitamin A concentrations in serum, and on terminal whole blood urea concentration of calves fed a whole milk diet [a]

	Treatments [b]								
	Mg	−	+	−	−	+	+	−	+	Standard
	T	−	−	+	−	+	−	+	+	deviation
Criterion	CLO	−	−	−	+	−	+	+	+	per calf
	(mg/100 ml. serum)									
Calcium		11.6	12.1	12.2	12.1	11.7	11.9	12.4	11.8	0.5
Inorganic phosphorus		8.7	9.9	8.8	8.4	9.5	9.9	8.7	10.5	0.6
Magnesium		2.16	2.61	2.29	1.03	2.79	2.60	1.71	2.50	0.27
Sodium		333	337	330	334	334	334	333	329	8
Potassium		20.8	19.8	19.8	21.8	20.4	22.4	19.9	21.1	1.0
Cholesterol		182	208	164	138	200	148	167	197	39
	(γ/100 ml. serum)									
Tocopherol		178	169	697	53	951	42	632	774	130
Carotenoids		48	59	36	7	40	11	22	21	11
Vitamin A [c]		25	23	27	34	22	31	33	28	7
	(mg. N°/100 ml. blood)									
Whole blood urea		8.1	9.0	7.7	9.4	8.0	10.6	8.6	7.8	1.3

[a] Values represent four calves per diet group and were obtained the 28th wk. of the supplement period.

[b] − indicates absence from the diet; + indicates presence.

[c] Adjusted by covariance (13) for initial values obtained the day prior to the addition of supplements to the diet.

APPENDIX TABLE III

Effects of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on heart and skeletal muscle dry matter and ash, heart and skeletal muscle (wet basis) calcium, phosphorus, magnesium, sodium, potassium, creatine, nitrogen, lipid, and tocopherol concentrations of calves fed a whole milk diet [a]

	Treatments [b]								
Mg	−	+	−	−	+	+	−	+	Standard
T	−	−	+	−	+	−	+	+	deviation
Criterion CLO	−	−	−	+	−	+	+	+	per calf
	(g/100 g. wet tissue)								
Dry matter									
Heart	22.0	22.2	21.5	20.6	22.1	20.5	23.2	22.5	0.4
Biceps femoris	23.6	23.9	23.5	22.3	23.3	21.9	23.5	23.6	0.4
Supraspinatus	23.1	23.0	23.0	21.8	22.4	21.6	22.6	23.0	0.4
Ash									
Heart	1.14	1.18	1.12	1.11	1.14	1.09	1.15	1.15	0.03
Biceps femoris	1.13	1.14	1.14	1.03	1.15	1.04	1.16	1.15	0.03
Supraspinatus	1.09	1.08	1.08	1.00	1.07	1.02	1.09	1.08	0.02
Nitrogen									
Heart	2.85	2.87	2.76	2.64	2.82	2.69	2.91	2.90	0.06
Biceps femoris	3.30	3.17	3.24	2.95	3.18	2.92	3.20	3.21	0.12
Supraspinatus	3.07	3.06	3.03	2.88	3.00	2.89	3.07	3.03	0.10
Lipids									
Heart	3.10	3.02	3.18	2.89	3.28	2.60	3.10	3.32	0.30
Supraspinatus	1.88	1.84	1.80	2.47	1.56	2.06	1.80	2.22	0.29
	(mg/100 g. wet tissue)								
Calcium									
Heart	3.2	3.0	3.4	8.9	3.6	6.0	3.1	3.2	1.3
Biceps femoris	3.7	3.6	3.7	5.6	3.4	5.3	3.7	3.6	0.2
Supraspinatus	3.1	3.2	3.3	5.5	2.7	4.8	3.0	2.9	0.4
Phosphorus									
Heart	238	239	230	205	233	205	238	238	5
Biceps femoris	215	216	215	184	216	186	216	219	5
Supraspinatus	204	207	203	184	202	192	203	206	5
Magnesium									
Heart	26.1	26.7	25.0	20.1	25.6	21.4	26.2	25.4	0.8
Biceps femoris	26.8	27.1	26.6	20.8	26.3	20.9	26.2	26.8	0.8
Supraspinatus	25.9	26.7	25.6	21.3	25.1	23.2	26.1	26.4	1.3
Sodium									
Heart	84	84	90	126	87	122	80	79	5
Biceps femoris	60	60	61	110	60	110	60	61	8
Supraspinatus	63	61	62	93	62	91	60	62	5
Potassium									
Heart	341	345	326	306	335	297	336	356	20
Biceps femoris	417	422	415	349	410	316	395	421	33
Supraspinatus	375	383	374	315	361	323	379	371	20
Creatine									
Heart	254	244	244	149	239	169	258	258	13
Biceps femoris	404	398	400	292	417	276	433	420	24
Supraspinatus	335	354	350	270	350	272	363	354	18
	(log γ/100 g. wet tissue)								
Tocopherol									
Heart	2.59	2.69	3.29	2.49	3.29	2.51	3.24	3.26	0.14
Supraspinatus	2.36	2.34	3.00	2.16	2.97	2.12	2.91	2.96	0.07

[a] Values represent four calves per diet group and were obtained at the completion of the 28-wk. supplement period.

[b] − indicates absence from the diet; + indicates presence.

APPENDIX TABLE IV

Effects of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on several biochemical constituents in livers of calves fed a whole milk diet [a]

	Treatments [b]								
	Mg	−	+	−	−	+	+	−	+	Standard
	T	−	−	+	−	+	−	+	+	deviation
Criterion	CLO	−	−	−	+	−	+	+	+	per calf
Liver										
Weight *(g.)*		2,634	2,644	2,768	3,297	2,627	3,472	3,003	2,826	313
Dry matter *(g/100 g.)*		26.3	26.1	26.8	25.9	26.4	25.6	26.2	27.0	0.8
Creatine *(mg/100 g.)*		72	64	63	59	63	61	59	59	7
Cholesterol *(mg/100 g.)*		287	318	270	261	273	248	270	261	33
Lipid *(g/100 g.)*		3.46	3.43	3.41	3.60	3.49	3.50	3.78	3.74	0.21
Tocopherol										
(γ/100 g.)		354	302	2,611	188	2,847	218	2,128	1,932	.....
(log γ/100 g.)		2.54	2.48	3.41	2.27	3.45	2.32	3.32	3.27	0.10
Carotenoids										
(γ/100 g.)		37	37	28	5	32	6	27	24	.....
(log γ + 1/100 g.)		1.57	1.56	1.46	0.73	1.51	0.69	1.45	1.39	0.21
Vitamin A										
(γ/100 g.)		1,490	1,386	1,708	6,608	2,197	6,370	28,599	27,846	.....
(log γ/100 g.)		3.16	3.10	3.23	3.82	3.33	3.80	4.45	4.43	0.12

[a] Values represent four calves per diet group and were obtained at the completion of the 28-wk. supplement period.
[b] − indicates absence from the diet; + indicates presence.

APPENDIX TABLE V

Effects of magnesium (Mg), tocopherol (T), and cod liver oil (CLO) on ash, calcium, and magnesium concentrations in bones of calves fed a whole milk diet [a]

	Treatments [b]								
	Mg	−	+	−	−	+	+	−	+	Standard
	T	−	−	+	−	+	−	+	+	deviation
Criterion	CLO	−	−	−	+	−	+	+	+	per calf
	(g/100 g.) [c]									
Ash										
Rib		56.8	58.1	56.9	58.4	57.6	57.6	57.4	56.9	1.3
Cannon		57.6	58.6	57.8	58.8	57.6	59.4	59.3	57.0	1.9
Tail		54.8	54.0	54.5	55.6	55.0	55.5	54.8	53.7	1.7
	(g/100 g. ash) [c]									
Calcium										
Rib		37.40	37.26	37.39	37.73	37.08	37.21	37.52	37.34	0.21
Cannon		37.29	37.16	37.12	37.70	37.06	37.06	37.24	37.02	0.25
Tail		37.39	37.07	37.44	37.80	37.04	37.08	37.42	37.10	0.12
Magnesium										
Rib		0.75	0.81	0.74	0.50	0.89	0.90	0.66	0.88	0.08
Cannon		0.69	0.79	0.72	0.43	0.82	0.82	0.62	0.83	0.08
Tail		0.64	0.75	0.67	0.40	0.79	0.79	0.57	0.79	0.06

[a] Values represent four calves per diet group and were obtained the 28th wk. of the supplement period.
[b] − indicates absence from the diet; + indicates presence.
[c] Lipid-free basis after extraction with ethyl alcohol and petroleum ether.

NITROGEN METABOLISM IN DAIRY CATTLE. I. THE INFLUENCE OF GRAIN AND MEADOW CROPS HARVESTED AS HAY, SILAGE, OR SOILAGE ON EFFICIENCY OF NITROGEN UTILIZATION

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SUMMARY

Efficiency of nitrogen utilization was studied in four experiments involving varying proportions of silage and hay, comparisons of silage and freshly cut meadow crop (soilage), effects of grain feeding, and feeding different ratios of roughage to grain. Feces and urine were collected separately and nitrogen balance and milk nitrogen determined. In all trials, lactating cows were allowed to eat roughage ad lib. No differences in nitrogen efficiency or urine losses were observed in cows fed different proportions of hay and silage. The nitrogen utilization response from freshly cut forages was different from that obtained with ensiled forages. Below 15% protein equivalent in the total ration, silage and soilage nitrogen were used with the same efficiency. Silage nitrogen declined in efficiency at higher levels, whereas soilage nitrogen was used as efficiently at the highest levels of protein equivalent measured as at the lowest. Adding grain to forage rations increased the efficiency of nitrogen utilization markedly, but increases in the grain content which raised the amount of grain from a 3:1 ratio of roughage to grain to a 2:1 ratio were without further effects.

Efficiency of nitrogen utilization is of particular interest in dairy cattle feeding trials. Fundamentally, cost efficiency in feeding dairy cattle has been based on the utilization of forages as the principal sources of nitrogen and energy.

That many dairymen were feeding higher levels of protein equivalent than could be used efficiently by dairy cows seemed probable, because of the continued trend toward increased production of high-nitrogen, more digestible roughages. This study was done with the present emphasis on efficiency of farm production in mind, in an effort to define more clearly the nitrogen status of dairy cows under conditions of free choice roughage consumption.

Practical feeding experiments and nitrogen balance studies are the common methods used in evaluating the protein of feeds. Feeding experiments have had the practical advantage of measuring the end results in terms of a salable product (milk). The classical studies on protein requirements were made by this method (5, 8, 9). However, they required a relatively long period of time and provided no information on the metabolism of nitrogen.

In nitrogen balance experiments the nitrogen consumed in the feed is determined along with the output nitrogen in the urine, feces, and milk in the case of lactating cows. These figures provide a measure of nitrogen metabolism and may be used to compute apparent digestibility and the efficiency with which nitrogen is used for productive purposes (4).

Critical levels of nitrogen intake which will keep the test animal in approximate nitrogen balance must be used when comparing the protein value of different kinds of feeds, and the results apply only for the physiological state of the

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experimental animal (1). On the other hand, when protein is fed in excess of requirements, unused nitrogenous compounds absorbed into the blood are excreted in the urine along with catabolized tissue nitrogen (3, 9, 12). Thus, nitrogen excreted in the urine is a useful tool for determining when and under what conditions excessive losses of nitrogen occur. It thereby serves as a measure of nitrogen efficiency.

In the following experiments the efficiency of nitrogen utilization and urine losses were studied in dairy cattle fed free choice different roughages varying in protein content. Other variables incorporated in this study were the amount of grain fed, proportion of silage to hay, and a comparison of freshly cut versus ensiled forage.

EXPERIMENTAL PROCEDURES

Apparent protein digestibility and nitrogen balance were determined in cows on various experimental regimes for collection periods of five or seven days. Dry matters were determined by oven drying, except for silage, which was determined by toluene distillation. The nitrogen content of the feed, feces, urine, and milk was determined, using the Kjeldahl procedure. In all cases fresh or refrigerated samples were used. Urine samples were preserved with hydrochloric acid or thymol.

Feed was sampled for analysis when weighed in to each cow twice daily. Refused feed was weighed and sampled at the A.M. feeding for analysis.

The quantitative collection of urine and feces, milking, and measurement of feed intake were carried out in the stalls usually occupied by the cows in the milking barn. The separate collection of urine and feces was facilitated by the use of rubber mats for cow beds; Sisalkraft paper strips, 4 by 5 ft., on which the feces were collected; and Gooch tubing urinals, similar to those described by Hansard (6), which were attached to small-mouthed metal milk pails. Urine collection pails were emptied twice daily. Electrical stall trainers were used to make the cows stand at the back of the stall for defecation.

Experiment 1. The objective of this experiment was to determine the efficiency of nitrogen utilization when legume-grass silage and hay were fed in different proportions. The hay and silage were harvested from alternate strips of the same fields. The nitrogen metabolism studies were carried out over a period of 3 yr. Grain was fed throughout the experiment in proportion to the amount of roughage consumed (3:1 ratio). The grain concentrate ration was composed of 450 lb. of corn, 300 lb. of oats, 100 lb. of wheat bran, 50 lb. of soybean oil meal, and 10 lb. of salt. The proportion of silage and hay fed, protein content of the roughages used, and the year in which the experiments were conducted are shown in Table 1.

The protein digestion and nitrogen balance trials were carried out with cows in their third and fourth months of lactation during the fourth, sixth, or eighth wk. of the 16-wk. feeding period.

Experiment 2. The nitrogen metabolism studies in this experiment were carried out to obtain further data on the effects of various levels of grain feeding when freshly cut (soilage) or ensiled meadow crops (silage) were fed.

TABLE 1

Proportions of hay and silage and protein content of the roughages fed in Experiment 1

Year	Cows	Trials	Silage	Hay	Protein content
	(No.)		(%)		
Forage 1					
1954	3	3	100	0	11.1
1954	3	3	80	20	11.1
1954	3	3	50	50	11.1
Forage 2					
1955	2	4	50	50	13.6
1955	2	4	20	80	13.8
1955	2	4	0	100	13.9
Forage 3					
1956	1	2	100	0	15.5
1956	1	2	50	50	15.1
1956	1	2	0	100	14.7

Digestion and nitrogen balance trials were carried out with Jersey cows fed each of the following regimes: Ration 1—Silage with no grain; Ration 2—silage with one-half grain; Ration 3—silage with full grain; Ration 4—soilage with no grain; Ration 5—soilage with one-half grain; Ration 6—soilage with full grain. The cows in the one-half grain blocks were fed 0.25 lb. of grain daily per pound of milk over 12 lb. The cows in the full grain blocks were fed 0.5 lb. of grain daily per pound of milk over 12 lb.

Five-day digestion and nitrogen balance trials were used. In 1957, two trials with one cow on each ration were carried out, beginning on May 25 and June 2. In 1958, a series of five digestion and nitrogen balances was completed, using one cow on each ration and starting with May 26 and at 4-wk. intervals thereafter through the summer. The soilage (freshly cut meadow crop) was chopped twice daily and sampled at the time of feeding. In the early summer, silage from the previous year was used, whereas the first cutting from the same year was used during the last part of the summer. The grain concentrate was composed of 66% corn and cob meal, 33% ground whole oats, and 1% iodized salt.

Experiment 3. This experiment was done to study further effects of minimum amounts of grain on nitrogen efficiency in milking cows fed soilage and changes in nitrogen metabolism with increasing plant growth and maturity.

Two cows were fed grain and soilage, and soilage alone was used as the control ration for two other cows. Grain was fed at the daily rate of ¼ lb. per pound of milk produced above 12 lb. daily. Three digestion trials were carried out on each regime during the summer feeding period. A mixture of equal parts of corn and cob meal and ground whole oats was used as the only grain concentrate.

Two pairs of cows fed soilage only were used on alternate weeks to determine the changes in apparent protein digestion and nitrogen utilization for a 5-wk. period starting May 23 and on the 42-day second- and 35-third-cutting aftermath growths.

Experiment 4. In this experiment the effects of feeding of two ratios (3:1 and 2:1) of roughage-to-grain on the efficiency of nitrogen utilization were

studied. Four cows which had been adjusted to legume-grass silage feeding over a 2-mo. period were divided into groups of two and fed either a 3:1 or 2:1 ratio of roughage to grain. After a 2-wk. preliminary period, the first of two digestion and nitrogen balance trials was begun. The legume-grass silage, predominantly alfalfa, had been ensiled after wilting. The amount of silage fed was varied according to the appetite of the individual cows, and the amount of grain fed within each group was adjusted daily to maintain a constant ratio of forage (dry basis) to grain. The only exception was that the quantity of silage and grain fed were held constant during the feces collection periods. Later, second-cutting alfalfa hay in early-bloom stage was used to replace all of the legume-grass silage in the ration, but the two ratios of hay to grain (3:1 and 2:1) were maintained. The digestion and nitrogen balance trials were conducted during the first and fifth week after alfalfa hay feeding was initiated.

The grain ration fed in both experiments was composed of 450 lb. of corn, 300 lb. of oats, 100 lb. of wheat bran, 50 lb. of soybean oil meal, 10 lb. of salt, and 10 lb. of bone meal.

RESULTS AND DISCUSSION

No notable differences in nitrogen efficiency or urine losses were observed in cows fed different proportions of hay and silage (Table 2). However, as will be noted later, differences would not be expected at the levels of protein used (11.6 to 14.8% total protein). The low apparent protein digestibility obtained in the third trial with the all-silage group was attributed to the high dry matter content of the silage (37 to 47%). Excessive heating caused some browning and apparently rendered the nitrogen less available.

TABLE 2

Efficiency of nitrogen utilization in dairy cows fed various proportions of legume-grass hay and silage containing different percentages of total protein [a]

Silage: hay ratio	Trials	Protein in ration	Protein digested	Urine nitrogen	Nitrogen balance	Milk production	Efficiency of nitrogen utilization [b]	
							Milk	Total
(%)	*(No.)*	*(%)*			*(g/day)*	*(lb/day)*	*(%)*	
Trial 1								
100:0	3	11.6	64.3	33.5	6.7	21.4	42.6	30.4
80:20	3	11.6	64.1	36.0	10.4	20.3	36.1	28.2
50:50	3	11.6	63.0	37.7	3.0	22.0	38.8	25.3
Trial 2								
50:50	4	13.6	68.2	45.1	1.8	27.7	37.6	23.8
20:80	4	13.7	69.4	42.7	2.7	26.6	36.9	26.1
0:100	4	13.7	64.4	44.2	−20.5	31.6	42.6	22.9
Trial 3								
100:0	2	14.8	56.4	30.2	22.1	21.6	37.4	29.4
50:50	2	14.5	63.8	28.0	48.7	32.5	33.6	35.7
0:100	2	14.2	69.9	39.5	23.8	21.9	31.5	30.6

[a] Grain fed in proportion to roughage consumed; 3:1 ratio of roughage (dry basis) to grain fed.

[b] Total nitrogen efficiency $= \frac{\text{Intake N} - (\text{urine N} + \text{fecal N})}{\text{Intake N}}$, Milk efficiency $= \frac{\text{Milk N}}{\text{Absorbed N}}$

Results of the silage vs. soilage experiments are summarized (Table 3). The data obtained in Experiment 3 using soilage only are presented also (Table 4). Nitrogen was used, in most cases, more efficiently when fed as soilage than as silage (Table 3). This is illustrated in a scattergram (Figure 1), in which total nitrogen efficiency obtained from individual nitrogen balances was plotted against the total protein equivalent of the ration. Forty-one trials in which silage was the only roughage fed are compared with 41 other trials in which soilage was fed. In no case were trials included in which the grain fed contributed more than 17% of the nitrogen. Thus, despite a variability in time of harvesting the two types of roughage, the 41 individual trials used are considered sufficient to be indicative of the general population of cows fed soilage and silage. As would be expected, the range in per cent of total protein in the soilage extended both lower and higher than was found in the ensiled forages, harvested at a time approximately optimum yield.

Silage nitrogen was used most efficiently when the content was below 15% total protein in the ration. Above this level efficiency dropped off sharply in the same way that efficiency has been shown to decline when the total protein content of the ration was raised by increasing the amount of protein concentrates (9). In contrast, nitrogen of legume-grass soilage was used at optimum efficiency at all protein levels measured up to 24.6%. This is of marked nutritional importance, since it is reflected both as additional yield in milk nitrogen and as retained nitrogen. The possibility exists that this difference in nitrogen utilization between

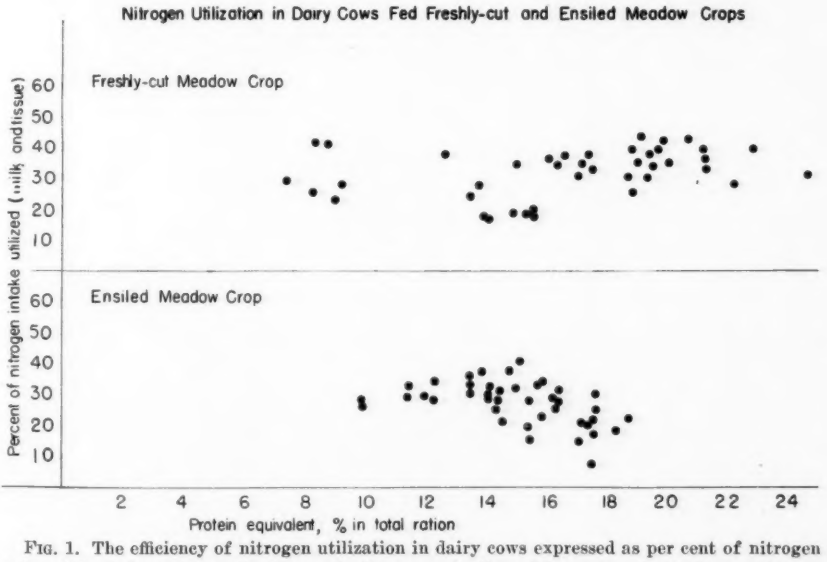

FIG. 1. The efficiency of nitrogen utilization in dairy cows expressed as per cent of nitrogen intake utilized for milk production and nitrogen retention. The upper chart shows nitrogen efficiency for 41 individual trials when soilage (freshly cut meadow crop) was fed, and lower chart shows 41 individual trials in which silage (ensiled meadow crop) was fed.

TABLE 3

Efficiency of nitrogen utilization in dairy cows fed meadow crop silage or soilage with different levels of grain concentrate

Roughage used	Amount of grain	Trials	Protein in ration	Protein digested [a]	Urine nitrogen [a]	Nitrogen balance [a]	Milk production	Efficiency of nitrogen utilization [a]	
								Milk	Total
		(No.)	*(%)*			*(g/day)*	*(lb/day)*	*(%)*	
Soilage	None	7	20.9	74.7	41.3	43.4	25.3	23.5	29.7
Soilage	One-half [b]	7	18.8	73.5	36.1	72.1	38.5	28.7	37.1
Soilage	Full [b]	7	18.1	72.0	36.2	64.6	28.7	27.3	35.9
Silage	None	7	16.8	63.2	44.1	−1.2	19.1	31.9	18.1
Silage	One-half [b]	7	15.7	64.6	37.8	22.6	25.7	31.0	27.3
Silage	Full [b]	7	15.3	63.9	33.9	17.5	23.2	33.3	29.7

[a] Difference between adjacent means required for statistical significance: protein digested, 5.4; urine nitrogen, 6.3; nitrogen balance, 30.7; efficiency of nitrogen utilization for milk, 9.3; and efficiency of utilization for total nitrogen, 7.2. Statistical Methods, p. 251 (14).

[b] Cows in groups receiving a full feed of grain were fed at the rate of 0.5 lb. of grain per pound of milk produced above 12 lb. One-half grain groups received grain at one-half the rate fed the full grain group.

TABLE 4

Efficiency of nitrogen utilization for various cutting dates in dairy cows fed freshly cut meadow crop with different levels of grain concentrate

Cutting date [a]	Amount of grain	Cows	Protein in ration	Protein digested	Urine nitrogen	Nitrogen balance	Milk production	Efficiency of nitrogen utilization	
								Milk	Total
	(lb.)	*(No.)*		*(%)*		*(g/day)*	*(lb/day)*	*(%)*	
May 28	None	2	15.5	68.8	50.7	−22.6	28.2	38.6	17.8
June 4	None	2	13.9	65.2	48.0	−21.6	29.4	40.6	17.3
June 11	None	2	12.8	65.1	25.5	15.4	24.5	48.7	39.9
June 18	None	2	9.1	59.4	34.4	−12.7	21.6	60.1	24.9
June 25	None	2	7.8	57.6	30.5	−13.4	18.9	77.7	27.1
July 30 [b]	None	2	17.3	74.1	40.3	77.0	22.4	21.0	33.8
August 20 [c]	None	2	18.8	75.8	49.0	54.6	21.8	17.6	27.2
May 28	4.7	2	15.0	68.5	42.3	−2.7	36.3	38.6	26.3
June 25	3.0	2	8.5	63.4	23.4	8.9	27.0	55.8	40.7
July 30	3.0	2	16.2	76.1	40.0	72.7	29.7	24.1	36.3

[a] Indicates the middle date of collection period.
[b] Aftermath forage, alfalfa in one-half bloom stage, approximately 42-day growth.
[c] Aftermath forage, alfalfa in bud stage, approximately 35-day growth.

TABLE 5

Efficiency of nitrogen utilization in dairy cows fed 2:1 or 3:1 ratio roughage (dry matter) to grain with legume-grass silage or alfalfa hay

Roughage used	Hay:grain ratio	Trials	Protein in ration	Protein digested	Urine nitrogen	Nitrogen balance	Milk production	Efficiency of nitrogen utilization	
								Milk	Total
		(No.)		*(%)*		*(g/day)*	*(lb/day)*	*(%)*	
Legume-grass silage	3:1	4	16.9	68.7	42.0	31.2	19.8	22.5	21.5
Legume-grass silage	2:1	4	16.8	69.9	46.0	16.7	23.9	27.4	23.9
Alfalfa hay	3:1	4	15.7	69.7	39.2	21.9	20.9	20.9	30.4
Alfalfa hay	2:1	4	15.4	66.9	38.4	20.0	25.5	25.5	25.9

TABLE 6

Multiple regression analysis.[a] Regression coefficients for independent effects of digestible dry matter intake, stage of lactation, nitrogen intake, and grain on milk nitrogen, urine nitrogen, and efficiency

	Dry matter	Lactation stage	Nitrogen	Grain [b]	Mean
	(lb/1,000)	*(days)*	*(lb.)*		*($\bar{y}$)*
SOILAGE					
Mean ($\bar{x}$)	20.96	109.49	.71	49%	
Milk N *(lb.)*	.0013	−.000344	.0452	.0263	.15
Urine N *(lb.)*	.0095	−.000023	.2917**	−.0531*	.28
Efficiency *(%)*	.332	.021	2.433	7.116**	31.5
SILAGE					
Mean ($\bar{x}$)	17.09	116.46	.55	81%	
Milk N *(lb.)*	.0018	−.000049	.1062**	.0139	.12
Urine N *(lb.)*	−.0127**	.000076	.5855**	.0030	.21
Efficiency *(%)*	1.616**	−.004	−31.470**	5.353	26.5

[a] ** Significant at .01—* Significant at .05.
[b] Grain present = 1, absent = 0.

silage and soilage reflected a difference in the nitrogen metabolism of the rumen caused by the high nonprotein nitrogen content of the meadow crop silage or, possibly, in the observed difference in types of rumen microorganisms found in the two groups of cattle (2).

The results presented (Table 3) show that cows fed freshly cut meadow crops stored very high amounts of nitrogen, even though they were in the early stages of lactation, in comparison with silage-fed cows. This higher nitrogen storage coincided with increased body weight gains. The physiological importance to the cow of replenishing the nitrogen lost through tissue catabolism during the early part of lactation merits further attention.

Grain added to the all-forage rations increased efficiency of nitrogen utilization markedly while decreasing urine losses (Tables 3 and 4). It is noteworthy that grain additions (ground corn and oats) tended to have a nonlinear effect. That is, addition of a small amount of grain effected a large increase in nitrogen efficiency but further additions failed to cause further increases. For example, cows fed at the one-half and one-fourth grain level, where grain intake ranged from 8 to 23% of the total dry matter, utilized nitrogen as well as cows fed the full amount of grain. Furthermore, the results of Experiment 4 (Table 5) show that nitrogen utilization for all nitrogen uses including milk production was equally as efficient in cows fed a 3:1 ratio of roughage to grain as in cows fed a 2:1 ratio.

A statistical study of the data was made to determine what effect each of various known variables had on nitrogen metabolism. The independent effects on milk nitrogen, urine nitrogen, and efficiency of stage of lactation, rate of feed intake (digestible dry matter per 1,000 lb. of body weight), total nitrogen intake, grain feeding (presence or absence), and type of forage used were assessed by computing a multiple regression on the silage and soilage data separately. The means and regression coefficients are shown (Table 6). It will be noted that, independent of the grain effect, the variations in dry matter consumed by cows

fed soilage had no significant effect on nitrogen utilization, whereas the dry matter consumed by the silage-fed cows was related to marked differences in efficiency ($P < .01$) and yield of milk nitrogen ($P < .05$). An explanation of this difference is seen by referring to the means of the digestible dry matter intakes. Twenty-one pounds of digestible dry matter per 1,000 lb. of body weight were consumed on the average by the cows fed soilage compared to 17 lb. when silage was fed. It is apparent, as others have observed (7, 13), that the cows fed silage failed to consume optimum amounts of roughage. This, in turn, limited the amount of energy available to utilize nitrogen and in some cases caused the cows to draw from their body tissue to the extent of throwing them into negative balance (Table 3). The differences in the effect on nitrogen utilization of soilage and silage, however, were not limited to dry matter intake. A difference in grain response to the two types of forage was noted (Table 6). Grain is shown to enhance the efficiency of nitrogen utilization independent of, or over and above, its effect on adding to the dry matter consumed. In the soilage-fed cows this effect was marked and highly significant, whereas with the silage-fed cows the effect was slight, nonsignificant, and could not be separated from the contribution it made through increasing the dry matter intake. This suggests that grain feeding may have two separate effects on nitrogen utilization, depending on the type and quantity of roughage consumed. First, increased efficiency may result from grain adding to the energy increment by augmenting the dry matter consumption and, secondly, by enhancing nitrogen utilization if the cows are already consuming maximum quantities of roughage energy.

In confirmation of Figure 1, a highly significant decline in the efficiency of nitrogen utilization in silage-fed cows receiving increasing levels of nitrogen is shown (Table 6). Essentially no relationship existed between amount of nitrogen intake and efficiency in cows fed soilage. As the nitrogen intake increased, urine nitrogen in the group fed silage increased twice the rate found for the soilage-fed group.

Apart from grain feeding, the multiple regression analyses (Table 6) showed that the variability in digestible dry matter intake was unrelated to efficiency within the group of soilage-fed cows. Maximum efficiency was obtained with grain feeding. In consideration of these two observations, and the general axiom that energy intake is positively related to nitrogen retention (11), the data from the animals in Experiment 2 were expanded, using the trials where simultaneous comparisons were made between soilage and silage. The total of productively used nitrogen, the amount retained plus milk nitrogen, was plotted against each cow's rate of energy intake in terms of digestible dry matter per 1,000 lb. of body weight (Figure 2). These data indicate that increased digestible dry matter intake also increased markedly the amount of nitrogen utilized daily by soilage-fed cows, even though the efficiency with which it was used remained relatively constant.

As shown in Figure 2, and pointed out in the foregoing discussions, the nitrogen-utilization-response from roughages fed as freshly cut forages is vastly different from that obtained with ensiled forages. Thus, at the highest level of

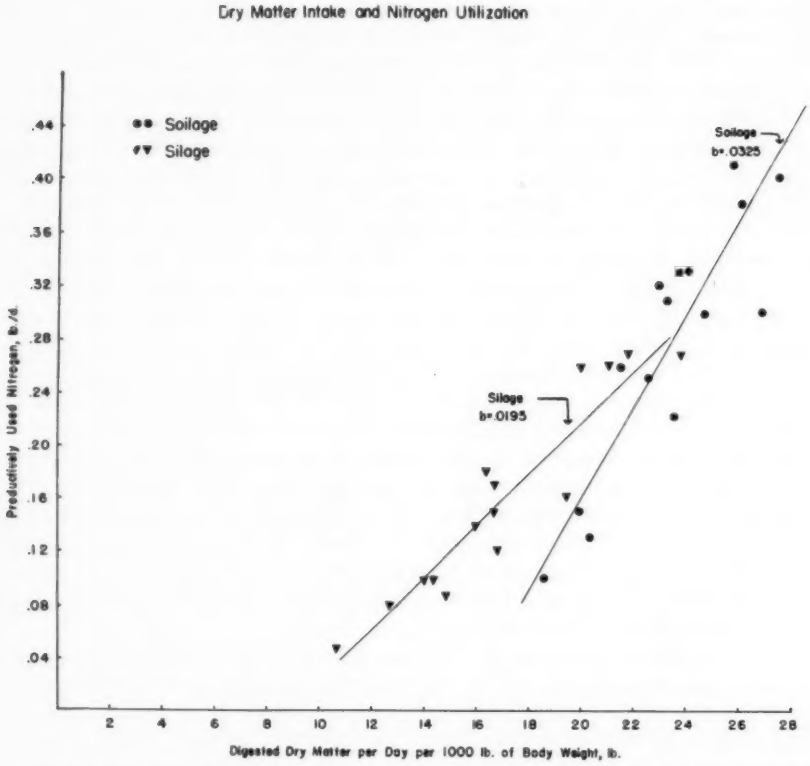

FIG. 2. The amount of nitrogen used by dairy cows at varying levels of dry matter intake with both silage and soilage when roughage consumption was voluntary.

digestible dry matter intake, the nitrogen in soilage was used most efficiently per unit of dry matter intake, whereas at the medium and lowest levels of digestible dry matter intake silage nitrogen was used most efficiently. This difference in nitrogen response in relation to digestible dry matter intake, reflected in the regression lines presented in Figure 2, offers a plausible explanation for the marked difference found among experiment station workers in comparative response of cows to hay and silage dry matter for milk production. For example, Stone *et al.* (15) and Kirsh and Jantzon (10) have found that ensiling enhanced both efficiency of dry matter utilization and milk production. Secondly, Pratt and Conrad (13) found efficiency of dry matter utilization to be increased in silage-fed cows compared to hay-fed animals, but total dry matter intake was less, resulting in approximately the same milk production. Thirdly, Hillman *et al.* (7) showed that the efficiency of dry matter utilization was highest for the silage-fed cows, but milk production was reduced by silage feeding.

Two points stand out in the practical application of these results. First, silage nitrogen was used most efficiently below 15% protein equivalent in the total ration and declined at higher levels of protein. Secondly, young cut forage fed as soilage was the most useful source of nitrogen studied, in that it was more efficiently used at higher protein levels than silage and at the same time allowed the cows to consume more digestible dry matter than was possible during silage feeding.

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GENETIC FACTORS AFFECTING CONCEPTION RATE AND EARLY PREGNANCY LOSS IN HOLSTEIN CATTLE [1]

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SUMMARY

Conception rate from first insemination and pregnancy loss to 152 days post-insemination were studied in 181 heifers and 346 parous cows. The effect of the sire line of the dam and system of mating of the embryo were studied in both groups and, in addition, the effect of season of birth on their conception rate was studied in the heifers. Adjustments were made to remove any effect of season of previous calving and parity on conception rate in the cows. Conception rate in the heifers was affected significantly by the season of birth and the interaction of sire line with the system of mating ($P<0.05$). In the cows, conception rate was affected significantly by the sire line ($P<0.01$) and the interaction of sire line with the system of mating ($P<0.05$). The over-all adjusted conception rate in the heifers was 63.6% as compared to 55.3% in the parous cows.

Pregnancy loss was not affected significantly ($P>0.05$) by either sire line or system of mating in the heifers, but the latter had a significant effect ($P<0.05$) on pregnancy loss in the cows. Higher conception rates were found in the systems of mating in which the potential embryo was outbred than those in which the potential embryo was inbred; whereas, pregnancy loss was greater in the systems of mating in which the dam was inbred than those in which the dam was outbred. This was true in both heifers and cows where the over-all losses were 2.5% in the heifers as compared to 13.0% in the cows.

The increased use of artificial insemination has made it possible to follow some selected lines of breeding. Presumably, this has led to some inbreeding and line-crossing, depending upon the plan or system of mating followed. Whether sire line or system of mating affects conception rate and pregnancy loss appreciably may be of considerable importance in selecting a mating plan. Woodward and Graves (16) reported a tendency for services per conception to increase as inbreeding was increased in a grade Holstein herd. Squires *et al.* (12) found significantly less embryonic death to 25 days in crossbred gilts than in one outbred and three inbred parental strains.

The heritability studies of breeding efficiency have not given consistent results. Studies by Dunbar and Henderson (3), Legates (8), and Pou *et al.* (9) reported heritabilities near zero, whereas Wilcox *et al.* (15) reported a heritability of 0.32 for calving interval and number of parturitions. Breeding

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efficiences of cow families ranging from 40 to 92% conception rate were reported by Seath *et al.* (10). Trimberger and Davis (14) found differences in cow families and sire families for conception rate ranging from 1.17 to 2.25 services per conception. Tabler *et al.* (13) reported no significant differences in breeding efficiencies among 19 cow families for services per conception, days from calving to first breeding, or days from first breeding to conception.

The object of the present study was to determine the effects of sire line and system of mating on conception rate and pregnancy loss to 152 days post-breeding in an experimental breeding herd.

MATERIALS AND METHODS

The data for this report were obtained between December 21, 1949, and December 20, 1955, from the Holstein-Friesian cows in the Emmons Blaine, Jr. Experimental Herd at Lake Mills, Wisconsin. A preliminary study of part of these same data was made by Hawk *et al.* (4), with the emphasis being placed on the effect of sire differences and inbreeding on estimated embryonic loss. The herd consists of six unrelated sire lines with four systems of mating, as was outlined in that report.

The sire line refers to the line of breeding of the sire of the cow (dam), and the system of mating refers to the genetic makeup of the potential embryo or fetus. The symbols used to designate the systems of mating are as follows: O-O, outbred embryo—outbred dam; O-I, outbred embryo—inbred dam; I-O, inbred embryo—outbred dam; and I-I, inbred embryo—inbred dam. In the statistical analyses, sire lines were considered to be representative of the Holstein population and thus treated as a random variable, while system of mating was considered as a fixed variable.

The data on heifers and parous cows were analyzed separately. Insufficient numbers of I-O matings occurred in the heifers during the time covered by this study; therefore, only three systems of mating are represented in the heifer data. The average inbreeding coefficients of the heifers in the O-I and I-I systems of mating were 27 and 23%, respectively, while the average inbreeding coefficient of the potential offspring in the I-I system of mating was 34%. The average inbreeding coefficients of the cows in the O-I and I-I systems of mating were 25 and 24%, respectively, with the average inbreeding coefficients of the potential offspring in the I-O and I-I systems of mating, 22 and 32%, respectively.

All animals were on a regular rectal palpation schedule as outlined by Kidder *et al.* (6). The standard procedures for culling cows from the herd were described by Buch *et al.* (2) and management practices responsible for maintaining environment as constant as possible were described by Buch *et al.* (1).

Conception rates were based on results of the first insemination of the service period. In the heifers, this insemination was at the first estrus on or after 15 mo. of age and, in parous cows, at the first estrus of each service period after 75 days postpartum, provided their reproductive organs were in a satisfactory breeding condition as determined by rectal palpation. The results of an insemination were determined by rectal palpation at 35–41 days post-insemination. The

presence of uterine contents, including palpable membranous material, was considered as evidence of conception.

The presence or absence of uterine contents was coded as 1 or 0, respectively. The analysis of variance for uterine contents in the heifers was made by the method of fitting constants (5). In the cows, it was made by the method of unweighted means (11), after adjusting to remove the effect of parity and season of calving.

The season of birth as used in the heifer analysis refers to the season in which the heifer was born: winter, December 21–March 20; spring, March 21–June 20; summer, June 21–September 20; fall, September 21–December 20.

Pregnancy loss was based on pregnancies that were diagnosed at 35–41 days post-insemination. In heifers, all diagnosed pregnancies that were lost by a heifer before one was carried at least 151 days contributed to a per cent loss as follows: $\frac{L}{L+1}100$ = per cent loss in heifer service period where L equalled the number of pregnancies lost before one was carried at least 151 days. This was done to prevent undue emphasis on any particular animal (some heifers lost as many as three pregnancies before carrying one or being culled from the herd).

In the parous cows, pregnancy loss was restricted to whether a cow lost or carried the first diagnosed pregnancy of her service period as follows: $\frac{L}{L+C}100$ = per cent loss in parous cows where L equalled the number of service periods in which the first pregnancy was lost and C the number in which it was carried at least 151 days. Thus, a cow could not lose more pregnancies than she had service periods.

In both heifers and cows, the percentage values were transformed to angles and the analysis of variance was made on the unweighted means of the angles for the lines and systems of mating.

RESULTS

Conception rate in heifers. The 181 first-service animals had an adjusted conception rate of 63.6%. From the analysis of variance (Table 1), season of birth of the heifer and the interaction of sire-line of the heifer with the system of mating of the potential embryo affected significantly the chance that a heifer would conceive to the first insemination. A multiple range test (7) for the effect

TABLE 1

Analysis of variance of heifers having uterine contents from first insemination by method of fitting constants

Source	D.F.	Error term	M.S.
Season of birth	3	R	0.82*
Sire line (L)	5	W	0.18
System of mating (S)	2	L × S	0.62
L × S	10	W	0.36*
Residual interaction (R)	44		0.27[a]
Within subclass (W)	116		0.19

* $P<0.05$.

[a] Composed of the mean squares of the possible interactions involving season.

TABLE 2

Multiple range test for season of birth of adjusted percentages of heifers having uterine contents from first insemination

Season of birth	Summer	Spring	Winter	Fall
Adjusted mean percentage	48.8	52.4	70.8	82.3

NOTE: Any two means underscored by the same line are not significantly different from each other at the 0.05 level of probability.

of season (Table 2) showed a significantly higher ($P<0.05$) conception rate in fall-born heifers (82.3%) than in spring- (52.4%) or summer-born (48.8%) heifers. Winter-born heifers had a conception rate that was intermediate (70.8%) and did not differ significantly from the other groups.

Illustration of the effect of the interaction of sire line with system of mating on conception in these heifers is presented in Table 3. Within the system of mating the sire line percentages had ranges of 17.4 for the O-I heifers and 73.3 for the I-I heifers. Within the sire-lines the system of mating percentages had ranges varying from 13.2 (Line 2) to 74.8 (Line 1).

Conception rate in parous cows. The 346 cows having service periods as parous individuals had a total of 513 first inseminations of the service period

TABLE 3

Adjusted percentages of heifers having uterine contents from first insemination

	Lines							
Systems	1	2	3	4	5	6	Av.	Range
O-O	92.2	65.7	81.0	56.5	53.7	66.7	69.3	38.5
O-I	77.8	60.4	64.3	73.5	75.6	73.5	70.8	17.4
I-I	17.4	73.6	90.7	36.6	45.0	40.7	50.7	73.3
Av.	62.5	66.6	78.7	55.5	58.1	60.3	63.6	
Range	74.8	13.2	26.4	36.9	30.6	32.8		

with an adjusted conception rate of 55.3%. The analysis of variance (Table 4) showed a significant ($P<0.05$) interaction of sire line with the system of mating, as well as a significant ($P<0.01$) sire line effect. Table 5 illustrates the effect of this interaction on conception rate in these animals. Within the O-O system of mating the sire line percentages had a range of 30.3, whereas within the three

TABLE 4

Analysis of variance of unweighted means of parous cows having uterine contents from first insemination of the service period

Source	D.F.	M.S.
Sire line (L)	5	.68**
System of mating (S)	3	1.27
L × S	15	.41*
Between cows	322	.22

*$P<0.05$.
**$P<0.01$.

TABLE 5
Adjusted percentages of parous cows having uterine contents from first insemination of the service period

Systems	Lines						Av.	Range
	1	2	3	4	5	6		
O-O	80.9	77.6	74.2	61.9	50.6	57.3	67.1	30.3
O-I	48.1	76.8	91.5	53.4	38.4	58.3	61.1	53.1
I-O	36.1	62.6	74.9	56.3	61.5	29.3	53.4	45.6
I-I	21.9	13.4	61.4	30.2	63.0	47.5	39.6	49.6
Av.	46.8	57.6	75.5	50.4	53.4	48.1	55.3	
Range	59.0	64.2	30.1	31.7	24.6	29.0		

other systems this range was from 45.6 to 53.1. Within the sire lines the range for system of mating percentages varied from 24.6 (Line 5) to 64.2 (Line 2).

Diagnosed pregnancy loss in heifers. The 181 heifers lost a total of 24 of the 198 diagnosed pregnancies. The analysis of variance in Table 6 showed that neither sire line nor system of mating had a significant effect on pregnancy loss in these animals. There is, however, a slight trend for an increase in loss for the inbred matings (Table 7, 1.3 and 2.1% for the O-I and I-I matings, respectively, as compared to 0.7% for the O-O matings). The over-all value of 1.3% would indicate the pregnancy loss in heifers to be relatively low.

Diagnosed pregnancy loss in parous cows. The 346 cows lost a total of 84 of the 494 first diagnosed pregnancies of their service periods. The analysis of variance showed the system of mating, but not the sire line, to affect pregnancy loss significantly. The greater losses (26.5 and 19.4% for the O-I and I-I matings, respectively) occurred in the matings in which the dam was an inbred, as compared to the matings in which the dam was an outbred (6.2 and 5.1% for the O-O and I-O matings, respectively). A further analysis on the combined unweighted means of the O-O with the I-O systems of mating (outbred dams) and the O-I with the I-I systems of mating (inbred dams) showed a significant

TABLE 6
Analysis of variance of heifers losing diagnosed pregnancies before 152 days post-insemination by method of unweighted means

Source	D.F.	M.S.
Sire line (L)	5	210
System (S)	2	175
L × S	10	426
Between heifers	161	395

TABLE 7
Heifers losing diagnosed pregnancies before 152 days post-insemination (percentages retransformed from angles)

Systems	Lines						Av.
	1	2	3	4	5	6	
O-O	0.8	0.0	2.7	5.7	0.8	0.0	0.7
O-I	0.5	2.4	0.0	1.7	0.0	10.9	1.3
I-I	3.8	0.6	4.9	0.0	1.2	6.7	2.1
Av.	0.7	0.6	1.7	1.5	0.4	3.9	1.3

TABLE 8

Analysis of variance of parous cows losing diagnosed pregnancies before 152 days post-insemination by method of unweighted means

Source	D.F.	M.S.
Sire line (L)	5	1,756
System of mating (S)	3	4,914*
L × S	15	981
Between cows	316	1,448

*$P<0.05$.

TABLE 9

Parous cows losing diagnosed pregnancies before 152 days post-insemination (percentages retransformed from angles)

	Lines						
Systems	1	2	3	4	5	6	Av.
O-O	5.2	12.7	4.4	9.9	5.0	2.5	6.2
O-I	16.3	20.6	25.6	6.7	85.4	14.6	26.5
I-O	6.7	1.7	1.5	4.0	9.2	10.8	5.1
I-I	19.6	29.2	9.6	8.4	34.6	20.6	19.4
Av.	11.2	14.1	8.4	7.1	30.5	11.1	13.0

difference in pregnancy loss between these two groups. Thus, it does not appear that the inbreeding of the fetus is an important factor in pregnancy loss during the stage of gestation studied, whereas the inbreeding of the dam may have a considerable effect.

DISCUSSION

Any factor affecting conception rate as used in this study either is interfering with the processes involved in fertilization or is causing embryonic death before such a time that an embryo or membranes would be detected at the pregnancy examination 35-41 days after insemination. Factors responsible for loss of pregnancies diagnosed as positive (although they might be abnormal) would be manifesting their effects by the animal returning to estrus or aborting before 152 days post-insemination. Thus, this study should disclose whether there is a differential effect of the factors studied on prenatal development prior to the pregnancy examination or subsequent to it.

Conception rate in both heifers and cows was affected significantly by the interaction of sire line with system of mating and, in addition, the main effect of sire line was significant in the cows. This would suggest the presence of different amounts of nonadditive gene action among the lines on conception rate in both heifers and cows, plus additive gene differences among the lines in the cows.

Pregnancy loss was affected significantly by the system of mating in cows, but was not affected significantly by any of the factors studied in the heifers. This would suggest the presence of nonadditive gene action on pregnancy loss in the cows, without evidence of the amounts differing in the various lines.

The magnitude of the effect of system of mating on pregnancy loss in cows was greater in the systems in which the dam was inbred (26.5 and 19.4% for the

O-I and I-I matings, respectively) than those in which the dam was outbred (6.2 and 5.1% for the O-O and I-O matings, respectively).

This was not the case in conception rate. For heifers the matings in which the potential embryo was outbred, the conception rate was about the same (69.3 and 70.8% for the O-O and O-I matings, respectively), as compared to 50.7% in the matings in which both potential embryo and dam were inbred (I-I). There was a similar trend in cows. The matings in which the potential embryo was outbred had higher conception rates (67.1 and 61.1% for the O-O and O-I matings, respectively) than those in which the potential embryo was inbred (53.4 and 39.6% for the I-O and I-I matings, respectively).

Thus, there is an indication that inbreeding of the potential embryo is more detrimental to the fertilization processes and/or early embryonic death than the inbreeding of the dam, whereas later embryonic and fetal loss are affected more by the inbreeding of the dam than by the inbreeding of the embryo or fetus.

From the over-all values, it appeared that conception rate from first insemination was somewhat higher in heifers than in cows (63.6 vs. 55.3%). However, when a comparison was made only in the outbred matings, the values were essentially the same (69.3 vs. 67.1%, for the heifers and cows, respectively). The lesser over-all value in the cows than in the heifers appeared to be due to the greater depression of conception rate in the I-I cows than in the I-I heifers. Also, the presence of the I-O system of mating in cows, which was not included in the heifers, was a contributing factor. Thus, the effect of inbreeding on lowering conception rate appears to increase following the heifer service period.

Pregnancy loss also was increased more by inbreeding in cows than in heifers. There was a greater loss in each system of mating represented in cows than in its counterpart represented in heifers. The difference between O-O and I-I in heifers was 1.4%, whereas in cows it was 13.2%. The over-all means were 13.0% for cows and 2.5% for heifers when both were calculated on the basis of first diagnosed pregnancy of the service period.

The significant effect of season of birth of a heifer on conception to first insemination may be due to season of a critical growth stage, season of insemination, or any other confounded factor, since these animals were bred at a constant age which could induce seasonal variations in all aspects of development from birth to conception.

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PART LACTATIONS. I. AGE-CORRECTION FACTORS FOR MONTHLY MILK FAT YIELDS

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SUMMARY

Age-correction factors are developed for monthly milk fat yields of dairy cows in New Zealand. The first test month of the lactation is taken into account, because in any calendar month cows are at different stages of lactation as well as at different ages. The factors obtained decrease as lactation progresses, indicating that in the later part of lactation the production of young cows is very similar to that of mature ones. This is probably due to the poor environment at this time of year masking the age differences. The factors for 2-yr.-olds differ greatly according to the first test month, but this effect is not apparent in the 3- and 4-yr.-olds, which vary little and are close to the factors for lactation production.

Factors are also presented for correcting for age and first test month simultaneously, so that monthly records of cows calving in different months can be grouped together. The means of records corrected by these factors show that longer-milking cows give greater yields not only because of being milked longer but also because they produced more each month. Cows starting lactation at a high level had, on average, longer lactations than those starting at a low level of production.

Age-correction factors are required for records of cows of different ages when making comparisons between cows, or using records for analytical studies to estimate genetic parameters. Such factors have been established on many occasions for lactation yields (1, 3, 4, 5, 9, 10), but no reports have been found of factors for individual monthly yields. Madden *et al.* (7) used correction factors that had been derived from 305-day lactation yields, and found they were "fairly suitable for correcting the center months but were in error for the extreme months." This paper presents factors that have been developed for Jersey cows in New Zealand to correct monthly milk fat yields for age. Factors are also developed for age and the cow's first month on test, so that the monthly records of cows calving at different times can be grouped together for statistical analyses to estimate genetic parameters of monthly production. These analyses will be reported subsequently.

PREPARATION OF DATA

Monthly records were collected for all cows in herds with some artificially bred daughters in production during the 1956-1957 and 1957-1958 dairy seasons. The records collected were the milk fat percentage and milk fat yield obtained from regular herd-testing figures, the combination of an evening's and morning's milking. The butterfat yield was calculated to the nearest 1 lb. from the butterfat percentage and the milk yield multiplied by 30 to bring it to a monthly equivalent. Only herds that were predominantly Jersey or Jersey-crossbred cows were included, and the cows of other breeds were omitted from these.

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Dairying is seasonal in New Zealand, with most cows calving in the spring months of July and August, so that ages are recorded to the nearest year only. The study was confined to cows aged 2-9 yr., those in the 5- to 9-yr.-old age group being considered as mature, as in (2) and (9). Herd testing starts in August each dairy season and cows first tested later than December were excluded, as were any that milked less than 100 days.

Sometimes a month's herd testing figures are missed from a cow's records. Such missing values were filled in by interpolation from the previous and succeeding months' figures. Values missing for the first month were inserted by extrapolation from the second month; 102% of the milk fat percentage and 92% of the butterfat yield, when the second month was September; 96 and 88%, respectively, for later months, these being gross comparison factors derived from the 1956-1957 data. Cows were omitted if more than 1 mo.'s missing values had to be replaced.

A general description of the data is given in Table 1, showing the lactation

TABLE 1
Lactation averages (pounds milk fat)

	1956-7		1957-8	
Age	Mean	No. of cows	Mean	No. of cows
(yr.)				
2	273	3,625	295	3,610
3	300	3,073	322	3,296
4	323	2,171	350	2,761
5–9	339	7,015	359	7,051
Number of cows:		15,884		16,718
Number of herds:		258		270

averages (pounds of milk fat) for the 32,602 cows in the complete study. The generally higher level of production in 1957-1958 compared to 1956-1957 as reported in (8), is reflected in this Table. The lactation yields were computed by adding the monthly yields, using a proportional amount of the first month's figures to cover the days from calving to 15 days after the test day. Drying-off dates are not recorded in New Zealand and the figures obtained in the last test period count for a whole month. Lactations exceeding 305 days were reduced to this length by deducting a proportion of the last month's production.

LACTATION LENGTH

Herd testing in each dairy season runs from August through May for the majority of herds in New Zealand and this is the period for which milk fat yields were studied. The mean lactation length of all cows tested 100 days or more is approximately 256 days (8), so there were many lactations shorter than 305 days. Consequently, there are not the same number of records in each calendar month. Furthermore, records in any calendar month are of different lactation months according to the cows' calving dates. The lactations were, therefore, grouped according to the first month on test.

TABLE 2

Number of records in each calendar month

Cows first tested in	Lactations No.	%	Number of records in Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
August	18,697	57	18,659	18,514	18,312	17,944	17,307	15,677	8,716
September	9,128	28		9,120	9,054	8,942	8,712	8,162	5,932
October	3,326	10			3,320	3,300	3,238	3,101	2,389
November	1,159	4				1,154	1,137	1,078	852
December	292	1					292	280	211
Total	32,602	100							

The number of records for each lactation month for these groups is given in Table 2. This shows that 57% of the lactations are from cows first tested in August, 28% from those first tested in September, and 10% from those first tested in October, a total of 95% first tested earlier than November, corresponding approximately to 94% cows calving by October 15 as reported in (8).

The figures in Table 3 are those of Table 2 grouped according to the age of

TABLE 3

Distribution of records according to age, first month tested, and calendar month

Age of cow	Cows first tested	No. of lactations	Per cent with records in Dec.	Jan.	Feb.	Mar.	Apr.	May
(yr.)								
2	Aug.	4,394	99	99	96	93	86	51
	Sept.	2,020		99	97	95	89	67
	Oct.	573			99	97	83	71
3	Aug.	3,670	99	98	95	90	81	45
	Sept.	1,750		99	97	94	86	62
	Oct.	643			99	97	93	71
4	Aug.	2,815	99	98	96	93	83	45
	Sept.	1,362		99	98	95	89	67
	Oct.	531				98	95	75
5–9	Aug.	7,818	100	98	96	93	84	46
	Sept.	3,996		99	99	96	91	65
	Oct.	1,579				98	93	71
All ages	Aug.	18,697	99	98	96	93	84	47
	Sept.	9,128		99	98	95	89	65
	Oct.	3,326			99	97	93	72

the cow. The groups first tested in November and December constituted such a small fraction (5%) of the sample that no further use was made of them. Table 3 shows the number of records each month as a percentage of the total in its group. Of those first tested in August, 51% of the 2-yr.-olds were milked in May, but only 45% of the older cows, indicating the higher persistency of young stock. These percentages also show that persistency is affected by the first month on test. For example, 93% of all August-starters were still being milked in their eighth month, March, whereas only 72% of the October-starters reached 8 mo., into May. The seasonal effects of the late summer and autumn had reduced the percentage milking for as long as 8 mo. when starting on test in October. This was true in all age groups.

TABLE 4

Lactation production according to first month on test

Age of cow	First month on test			
	August	September	October	All 3 months
(yr.)	Mean milk fat *(lb.)*			
2	288	279	247	282
3	312	310	293	310
4	340	333	320	336
5–9	351	347	324	347
	Multiplicative age-correction factors			
2	1.22	1.25	1.31	1.23
3	1.12	1.12	1.10	1.12
4	1.03	1.04	1.01	1.03

LACTATION AVERAGES

The top section of Table 4 shows the mean lactation milk fat production grouped according to the cow's age and its first month on test (August, September, or October). The average production is also shown for the age groups regardless of first month on test. The greatest drop in production associated with late calving is in the 2-yr.-olds, the August-starters averaging 288 lb. and the October-starters 247 lb. The 3- and 4-yr.-olds show only half this drop, and the 5- to 9-yr.-olds starting in October gave 27 lb. less than those starting in August. Variations in lactation length may partly account for these differences, because September- and October-starting cows can not have the long lactation available to August-starting cows, since all lactations are taken as ending in May.

Multiplicative age-correction factors evaluated by the gross comparison method from a body of contemporary records, Method A of Lush and Shrode (6), are of the form

$$\frac{\text{mean yield of mature cows}}{\text{mean yield of young cows}}.$$

These factors for lactation yield are shown in the lower section of Table 4. The effect of first month on test is negligible for the 3- and 4-yr.-olds, but for the 2-yr.-olds the factors increase with later starting; i.e., the age correction for 2-yr.-olds to bring them up to the level of mature cows is greater for cows starting on test in October (1.31) than for those starting in August (1.22). This is because the difference between October- and August-starting cows is greater for 2-yr.-olds than for mature cows. Age-correction factors are, therefore, greater. The factors for all 3 mo. combined are almost identical to those being used at present (9). They are similar to the factors for August-starting cows, these being more than 60% of the sample (Table 3).

MONTHLY AVERAGES

The average milk fat yield each month is shown in Table 5, grouped according to the cow's age and first month on test. These are averages

TABLE 5

Average monthly production

Age of cows	First month on test	Monthly yield (pounds milk fat)									
		Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
(yr.)											
2	Aug.	31	35	37	37	35	32	27	26	24	21
	Sept.		34	39	39	38	35	30	27	26	22
	Oct.			37	40	39	35	30	27	27	23
3	Aug.	37	40	42	40	38	35	29	27	25	21
	Sept.		40	45	47	42	39	33	29	27	23
	Oct.			46	48	46	42	35	32	29	24
4	Aug.	41	44	45	43	41	37	31	28	26	21
	Sept.		44	49	48	45	41	35	31	29	23
	Oct.			50	53	50	45	39	34	31	25
5–9	Aug.	43	45	47	45	43	39	32	29	26	21
	Sept.		45	51	50	47	43	37	33	29	24
	Oct.			50	54	51	46	39	35	33	26

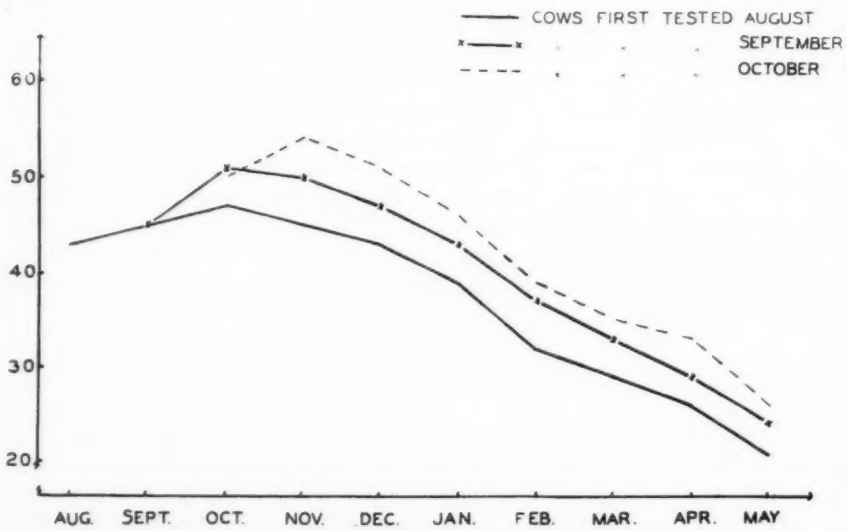

FIG. 1. Monthly milk fat production of mature cows (5- to 9-yr.-olds).

over the 2 years 1956-1957 and 1957-1957. The values for each year separately followed the same pattern as these, 1957-1958 being greater than 1956-1957, as indicated in Table 1. The lactation curves for the mature cows in Table 5 are shown in Figure 1; curves for the other ages are similar to these.

These monthly averages follow the well-known pattern of lactation curves, although at a lower level of production than that reported by Madden *et al.* (7). The peak of production is the third month of lactation for cows first tested in August, and the second month for September- and October-starting cows. This

corresponds with the good feed supply available in October and November, due to the spring flush of pasture. The later-starting cows are higher in production each month than the early-starting ones, throughout the whole lactation. This is because in each calendar month the September- and October-starting cows are at earlier stages of their lactations than the August-starters and in the first few months of lactation they are starting lactation with a better supply of feed. The older cows have higher yields than the young ones over most of the lactation, but by May this age difference is greatly reduced, although the difference due to first month on test still remains. There is a noticeable drop in production from January to February for all cows, less for the 2-yr.-olds than the mature cows. The later a cow started on test the larger the fall in production, i.e., the nearer the peak of production is to February, the larger is the fall in production. This probably arises from the shortage of feed at this time, when temporary drought conditions may occur and grass growth is retarded. As the autumn progresses through March and April into May, production declines still further but at a slower rate.

These averages are based on differing numbers of cows; namely, the cows on test in each particular month. Thus, the 21-lb. average in May of the 5- to 9-yr.-olds first tested in August is the average of 46% of the 7,818 lactations in that category (Table 3), i.e., of 3,573 records.

CORRELATION FACTORS

(1) For age. The first month on test had to be taken into account when age-correcting monthly records, because in any calendar month cows are at different stages of lactation as well as at different ages. The factors were calculated on a within starting-month basis so that both the young and the mature cows contributing to a factor were at the same stage of lactation. For example, in October the August-starting cows are in their third month of lactation and the 2-yr.-olds among them were compared with the October production of the August-starting mature cows; i.e., the multiplicative age-correction factor for the October production of 2-yr.-olds starting in September is

$$\frac{\text{mean October yield of mature cows starting in September}}{\text{mean October yield of 2-yr.-olds starting in September}}$$
$$= 51/39, \text{ from Table 5,}$$
$$= 1.30.$$

Factors calculated this way for the August-, September-, and October-starting cows in each age group are shown in Table 6. These factors have been taken to two decimal places; in use they are applied to monthly productions recorded to the nearest pound of fat. With the average monthly fat yield being about 44 lb. it takes a difference of 0.02 in a factor to cause a difference of 1 lb. of fat in a corrected record. A monthly yield is approximately 10% of a yearly yield, so that taking the standard deviation of yearly yields as 60 lb. that of monthly yields is approximately 6 lb. Therefore, a difference of 1 lb. of fat in a corrected monthly record caused by a difference of 0.02 in a correction factor is negligible. Bearing this in mind, this table shows:

TABLE 6
Multiplicative age-correction factors for monthly milk fat yields

Age of cow	First month on test	Age-correction factor									
		Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
(yr.)											
2	Aug.	1.36	1.30	1.27	1.23	1.21	1.20	1.18	1.15	1.06	1.00
	Sept.		1.32	1.30	1.27	1.27	1.23	1.22	1.20	1.14	1.09
	Oct.			1.34	1.34	1.32	1.32	1.29	1.28	1.23	1.17
3	Aug.	1.15	1.13	1.12	1.12	1.12	1.12	1.12	1.10	1.06	1.01
	Sept.		1.12	1.12	1.11	1.12	1.12	1.11	1.12	1.08	1.04
	Oct.			1.08	1.11	1.11	1.11	1.10	1.10	1.12	1.11
4	Aug.	1.02	1.03	1.03	1.04	1.04	1.05	1.03	1.02	1.01	0.99
	Sept.		1.02	1.03	1.05	1.05	1.04	1.04	1.04	1.03	1.05
	Oct.			1.00	1.01	1.03	1.02	1.01	1.01	1.04	1.04

(a) As lactation progresses the factors decrease, getting very close to 1.00 in the later months, indicating that in the later part of lactation the production of young cows is very similar to that of mature ones. This can probably be ascribed to the poor environment in April and May, masking the age differences.

(b) The factors for the last part of a 2-yr.-old's lactation are less than those for a 3-yr.-old at the start of lactation. This is in line with well known higher persistency of 2-yr.-olds compared with mature cows and consequent similarity to them at the end of lactation; when starting the next lactation, as 3-yr.-olds, age differences are again apparent and the correction factor, therefore, exceeds that at the end of the 2-yr.-old's lactation.

It is interesting to speculate on what this means in terms of the effects of the dry period following the 2-yr.-old lactation. A mature cow is building up condition for calving and the next lactation during the dry period, whereas a 2-yr.-old is also growing and undergoing body development. This would appear to contribute less towards the next lactation than the rebuilding of condition, particularly in New Zealand, where 2-yr.-olds usually finish out their lactations in relatively low condition. This is seldom fully recovered before calving, apparently because some of the dry period benefits are going towards growth and development. Hence, the young cows at the start of their 3-yr.-old lactation appear lower in production relative to the mature cows than they did at the end of their 2-yr.-old lactation. Accordingly, the correction factor for the first part of a 3-yr.-old's lactation exceeds that for the end of a 2-yr.-old's lactation.

(c) The factors for the 2-yr.-olds differ greatly according to the month of starting on test, but this effect is not apparent in the 3- and 4-yr.-olds.

(d) The factors for the 3- and 4-yr.-olds do not vary greatly from month to month, and are mostly fairly close to the factors for lactation production in Table 4. However, the factors for the 2-yr.-olds vary quite considerably from the 1.23 for lactation yield. Using this for the monthly yields would under-correct the early months and over-correct the late ones, but be more or less correct for the center months as suggested by Madden *et al.* (7).

(2) For age and first month on test. Statistical analyses of monthly yields would have to be done on each first-month-on-test group separately if the records

were age-corrected by the above factors. The age-corrected records could not be combined by calendar months because this would combine records made in different lactation months. Likewise, the data could not be combined by lactation months because this would put records together that were made in different calendar months. Hence, factors were developed to correct the records for both age and calendar month simultaneously. All records were corrected lactation month by lactation month up to the level of the mature cows that started in August. This meant that corrections were made to mature cows starting in September and October, as well as to the young cows. For example, the multiplicative correction factor to correct the October production of 2-yr.-olds starting in September is

$$\frac{\text{mean September yield, of mature cows, starting in August}}{\text{mean October yield, of 2-yr.-olds, starting in September}} = 45/39 \text{ from Table 5,}$$
$$= 1.15.$$

October is the second lactation month of the September-starting 2-yr.-olds, so these records were corrected up to the level of the second lactation month of the August-starting mature cows, which is September. These multiplicative factors are shown in Table 7. The following comments arise from Table 7:

TABLE 7

Multiplicative factors to correct monthly milk fat yields for age of cow and first month on test

Age of cow	First month on test	Correction factor for age and first month on test									
		Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
(yr.)											
2	Aug.	1.36	1.30	1.27	1.23	1.21	1.20	1.18	1.15	1.06	1.00
	Sept.		1.24	1.14	1.18	1.21	1.22	1.29	1.19	1.13	1.18
	Oct.			1.14	1.13	1.20	1.28	1.40	1.42	1.23	1.31
3	Aug.	1.15	1.13	1.12	1.12	1.12	1.12	1.12	1.10	1.06	1.01
	Sept.		1.05	1.00	1.03	1.06	1.10	1.18	1.11	1.08	1.13
	Oct.			.92	.93	1.02	1.08	1.20	1.22	1.12	1.23
4	Aug.	1.02	1.03	1.03	1.04	1.04	1.05	1.03	1.02	1.01	.99
	Sept.		.96	.92	.97	1.00	1.03	1.10	1.04	1.03	1.14
	Oct.			.85	.85	.94	1.00	1.10	1.12	1.04	1.16
5–9	Sept.	.....	.94	.89	.93	.95	.99	1.06	1.00	.99	1.08
	Oct.			.85	.84	.91	.97	1.09	1.11	1.00	1.11

(a) The factors are affected by the cow's first month on test, as would be expected, since they are designed to correct for this effect as well as age.

(b) The factors for the August-starting young cows are pure age-correction factors (as in Table 6) and they follow a decreasing trend as lactation continues. The factors for the September- and October-starting cows show no such trend. The factors for the mature cows correct for calendar month alone.

(c) Many of the factors are less than 1.00, indicating that the actual records have to be reduced to correct them to the level of mature cows starting in August. This is because later-starting cows begin their lactations in better con-

dition than early-starting ones and so their monthly yields are higher when compared on a lactation month basis.

The purpose of these factors is to correct monthly yields for age and calendar month so that records of all cows can be combined on a lactation month basis for statistical analyses. Hence, for example, the November yield of a 2-yr.-old starting in August would be multiplied by 1.23 and the December yield of a 3-yr.-old starting in September would be multiplied by 1.06; the corrected records would be records in the fourth lactation month, corrected for age and calendar month. The correction factors have the effect of bringing records up to the level of 5- to 9-yr.-old cows first tested in August.

TABLE 8

Rate of change of monthly yield of 2-yr.-olds, and of mature cows starting in August; monthly yield as a percentage of that of previous month

Age	First month tested	Calendar month of record								
		Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
(yr.)		*(%)*								
2	Aug.	111	106	100	96	92	85	92	96	85
	Sept.		115	101	95	93	86	91	96	85
	Oct.			107	97	91	86	90	97	85
5-9	Aug.	106	104	97	94	91	84	89	89	80

Some explanation of the trends in these factors can be gained from Table 8, which shows the rate of change in monthly production for 2-yr.-olds, and mature cows starting on test in August. These figures are the proportion which the yield in each month bears to that of the preceding month. At the start of lactation monthly yields are increasing (percentages greater than 100) and the proportional increases in yield, month to month, of the 2-yr.-olds are more than those of the mature cows. In the later stages of lactation monthly yields are decreasing and the proportional decreases in the 2-yr.-olds, month to month, are less than those in the mature cows. This causes the correction factors for the 2-yr.-olds commencing in August to decrease steadily. In effect, the 2-yr.-old is more persistent than the mature cow; both are affected by the deteriorating environment at the end of the season, but this shows up more in the mature cows than in the 2-yr.-olds, which produce progressively more like the mature cows at the end of lactation.

The factors for the September-starting cows are calculated by comparing the 2-yr.-olds' yields with those made by the mature cows one calendar month earlier. As with August-starting cows, the big increase in the 2-yr.-olds from first to second month of lactation leads to a decrease in the multiplicative factor. But, as the lactation progresses, the worsening environment is encountered a month earlier in lactation than with the August-starters, and the proportional drop in lactation from month to month is larger than with the mature cows of the previous month, so that the multiplicative factor increases over the third, fourth, and fifth months. However, at the end of the lactation the 2-yr.-old is once again producing more like a mature cow, the effect of September-starting has worn off, and the correction factor decreases.

The same trends occur in the factors for the 2-yr.-olds starting in October and, generally speaking, the 3-yr.-olds and 4-yr.-olds behave in a similar manner.

USE OF FACTORS

Correction factors should be used on data other than those from which they are developed. The data were insufficient in this study to develop factors from one half for use on the other half, so they were calculated from, and then applied to, all the data. The multiplicative factors were converted to

TABLE 9

Mean corrected record according to number of months tested

No. of months tested	No. of cows	Mean corrected record in lactation month									
		1	2	3	4	5	6	7	8	9	10
4	119	32	30	30	26						
5	176	33	32	32	30	25					
6	360	37	36	36	34	30	24				
7	992	39	39	42	40	37	31	23			
8	3,206	41	42	45	43	41	36	30	23		
9	6,058	42	44	46	45	43	39	33	28	23	
10	4,242	42	44	47	47	45	41	35	30	27	21

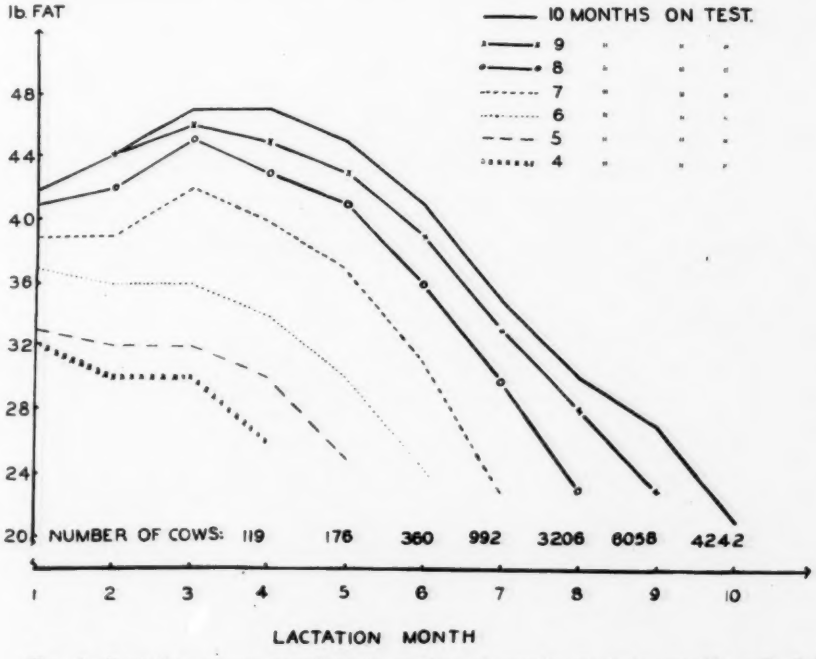

FIG. 2. Lactation curves according to number of months on test; monthly milk fat yields corrected for age and first month on test.

herd-level factors by the method outlined in (9); namely,

$$\text{herd level factor} = 1 - 1/(\text{multiplicative factor}).$$

The corrected herd average for each lactation month was calculated as

$$h = \frac{T}{N - \Sigma n_{ij} p_{ij}}$$

where T is the total actual production in the herd for that lactation month and N the number of cows, and n_{ij} and p_{ij} are the number of cows and herd-level correction factor, respectively, appropriate to i-yr.-olds first tested in month j, j being August, September, or October. The additive correction for the n_{ij} cows is then $p_{ij}h$. The lactation yields were corrected for age by the herd-level factors 0.23, 0.13, and 0.03 in regular use (9).

The means of the corrected monthly records for 1956-1957 are shown in Table 9, the cows being grouped according to the number of months on test. The corrected records in a lactation month, from which calendar month effects have been eliminated, increase as the number of months tested increases, showing that longer-milking cows give greater yields not only because of being milked longer but also because they produce more each month. This is seen clearly in Figure 2, which shows the lactation curves drawn from Table 9. Generally speaking, cows that start off as high producers give greater yields each month and dry off later than the cows that start their lactations at a low level of production.

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INTERRELATIONSHIPS BETWEEN CERTAIN MEASUREMENTS OF EXTERNAL BODY FORM, INTERNAL ANATOMY, AND FAT PRODUCTION

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SUMMARY

Correlations between butterfat production and certain measurements of external body form were calculated from data on 216 first-lactation Holstein cows in the Beltsville herd. The correlations between production and cross-section areas of chest or paunch were lower, in most cases, than those between production and other chest or paunch measurements. They were 0.18 between production and area of chest and 0.23 between production and area of paunch. Measurements most highly correlated with production were body length (0.31), height at withers (0.35), and depth of paunch (0.36). Multiple correlation coefficients between different sets of measurements and fat production did not exceed 0.40.

Measurements of external and internal anatomy were studied on 146 Holstein cows that had been measured before and after slaughter. Depth of chest was significantly correlated with internal chest depth (0.69), heart weight (0.57), and lung weight (0.32); depth of paunch with liver weight (0.48), stomach weight (0.38), length of small intestines (0.31), length of large intestines (0.37), and length of total intestines (0.36). Height at withers showed significant correlations with heart weight (0.50) and lung weight (0.28); body length with heart weight (0.49) and length of intestines (0.34). Partial correlations, with empty body weight held constant, showed that the correlations between certain external and internal measurements were a result of the common association with body weight.

The relationship between size of internal organs and fat production was studied on 115 Holstein cows. Correlations were significant only in the case of length of large intestines (0.29) and length of total intestines (0.25). The correlation between depth of paunch and butterfat production was partitioned into a direct component and a component contributed by the association between depth of paunch and organs of the digestive tract. The contribution of the direct path from depth of paunch to fat production amounted to at least 35% of the total correlation. The findings do not support a general supposition that correlations between external body measurements and production are a result of interrelationships with size of internal organs.

Many workers have investigated associations between body measurements and milk or butterfat production in dairy cattle. A large number of measurements have been studied and the literature on this subject has been reviewed by Heidhues (2) and Johnson (3). Results to date indicate that there are positive associations between milk or fat production and certain body measurements such as height at withers, body length, and head length. The present analysis was made to determine: (1) the associations between cross-section area of chest and paunch and fat production, (2) the multiple correlations between groups of body measurements and fat production, and (3) the interrelationships between external measurements, internal anatomy, and fat production.

Description of data. The data for this analysis were obtained over a period of 28 yr. in the Holstein-Friesian herd at Beltsville. This herd was maintained

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for a breeding project to investigate the genetic gain in production that might be made by the use of out-breeding with proved sires. Occasionally, sons of these sires were produced and used in the herd. Sires were selected with chief emphasis on butterfat production. Selection of the cows on the basis of production was avoided. Insofar as possible, uniform environmental conditions were maintained over the years.

About 30 body measurements were taken routinely at different ages, one of which was 3 mo. after first calving. Also, a large number of animals were measured shortly before slaughter (ante-mortem). Internal measurements were taken on these animals after slaughter (post-mortem). In addition to the cross-section areas of chest and paunch, several other external measurements were used in this study. These were selected from measurements having the highest correlations with production as reported in other studies. They included body weight; height at withers; depth, width, and circumference of chest; depth and circumference of paunch; body length (withers to pinbones), and length from withers to hips. Contours of chest and paunch were obtained by carefully fitting a flexible, rubber-covered lead bar to fit the shape of the animal. From this bar the contour lines were drawn on a sheet of paper, and the areas included in the drawings were measured with a planimeter (7).

Body capacity is emphasized in dairy cattle judging as an indication of producing ability. Therefore, the sum of both cross-section areas was taken as an estimate of body capacity, and the correlation with fat production was calculated. In another analysis, body length from withers to hips was taken into account in addition to the cross-section areas, and body capacity was calculated as

$$\frac{\text{area of chest} + \text{area of paunch}}{2} \times \text{length withers to hips.}$$

Only those post-mortem measurements which could be assumed to be associated with the external measurements used were selected for this study. This was necessary because it was not feasible to include all of the measurements that were taken. Those selected were depth of thoracic cavity at the seventh vertebra, width of thoracic cavity at the sixth rib, heart weight, lung weight, liver weight, stomach weight, length of large intestines, length of small intestines, length of total intestines, and empty body weight. Empty body weight was calculated as the difference between the weight immediately before slaughter and the weight of the contents of stomach and intestines.

All animals with measurements of the cross-section areas of chest and paunch and a first-lactation butterfat record were used for this analysis. The cows were divided into three groups according to degree of inbreeding. The first group was outbred animals, the second group was inbred animals with inbreeding coefficients of 12.5% or less, and the third group was animals with inbreeding coefficients exceeding 12.5%. These groups will be referred to as O, I_1, and I_2, respectively. The correlation coefficients for these groups were transformed to z values, weighted for degrees of freedom within groups, and tested for differences between them. Since no significant differences were found, the correlations were combined into a common estimate (5).

TABLE 1
Means, standard deviations, and coefficients of variation for butterfat production and various body measurements

	Mean	Standard deviation	Coefficient of variation
Butterfat production *(lb.)*[a]	498	121	24.3
Depth of chest *(cm.)*[a]	70	3.1	4.4
Depth of paunch *(cm.)*[a]	72	2.9	4.0
Height at withers *(cm.)*[a]	134	3.4	2.5
Body length *(cm.)*[a]	136	4.4	3.2
Length withers to hips *(cm.)*[a]	92	3.2	3.4
Depth of thoracic cavity *(cm.)*[b]	48.9	2.0	4.1
Width of thoracic cavity *(cm.)*[b]	32.4	1.9	5.9
Area of chest *(sq. cm.)*[a]	2,301	194	8.4
Area of paunch *(sq. cm.)*[a]	3,707	329	8.9
Circumference of chest *(cm.)*[a]	187	6.8	4.6
Circumference of paunch *(cm.)*[a]	225	9.2	4.1
Body weight *(lb.)*[a]	1,170	106	9.0
Empty body weight *(lb.)*[b]	1,101	156	14.2
Liver weight *(lb.)*[b]	15.9	2.4	15.1
Length of small intestines *(m.)*[b]	48.3	4.5	9.3
Length of large intestines *(m.)*[b]	13.0	1.3	10.0
Length of total intestines *(m.)*[b]	61.3	5.4	8.8
Stomach weight *(lb.)*[b]	37.6	5.3	14.1
Lung weight *(lb.)*[b]	9.2	1.3	14.1
Heart weight *(lb.)*[b]	4.9	0.6	12.2

[a] Obtained on first-lactation animals.
[b] Obtained from animals in post-mortem group.

Results and discussion. Means, standard deviations, and coefficients of variation for production and measurements are shown in Table 1. The variation in butterfat production is considerably greater than in any of the measurements. Three groups of body measurements may be considered. The first one includes height, length, and depth measurements, which are indicators of skeletal size. The variation of these items is relatively small as compared to the other measurements. The second group is comprised of measurements largely influenced by the condition of the animals, i.e., body weight and cross-section areas of chest and paunch. The third group, internal organs, has the greatest variability.

Phenotypic correlations. Correlations between first-lactation, 3 × 365-day actual records and external body measurements were calculated on a within sire, within year basis. The measurements were taken approximately 3 mo. after first calving. When the data were arranged in these subgroups, 216 animals were available with 152 in the O group, 40 in I_1, and 24 in I_2. Seventeen sires were represented and the total number of subgroups was 55. Production records were not adjusted to a single age since there was no feasible way to adjust the body measurements for age differences. Also, there was little variation in age at first calving; 180 of the 216 animals were between 23 and 28 mo. old at first calving. The correlations are given in Table 2. Those between the cross-section areas and production generally are lower than the correlations between circumference and depth of chest or paunch and production. Therefore, these areas are less useful than other chest or paunch measurements in estimating producing ability. Furthermore, the correlations between cross-section areas and the respective circumferences were high, being 0.93 between cross-section area and circumfer-

TABLE 2
Correlations between body measurements and butterfat production

Measurements	r
Cross-section area of chest	0.18*
Cross-section area of paunch	0.23**
Circumference of chest	0.23**
Depth of chest	0.24**
Circumference of paunch	0.22**
Depth of paunch	0.36**
Height at withers	0.35**
Body length	0.31**
Length, withers to hips	0.28**
Body weight	0.22**
Body capacity (area of chest + area of paunch)	0.22**
Body capacity $\left(\frac{\text{area of chest + area of paunch}}{2} \times \text{length, withers to hips}\right)$	0.28**

* $P<0.05$.
** $P<0.01$.

ence of chest, and 0.98 between cross-section area and circumference of paunch. The correlations between other body measurements and production were comparable to those obtained by Johnson (3) from a different sample of the same herd.

Body weight has been used frequently as a measure of size. However, it may be affected considerably by the condition of the animal. On the other hand, measurements of skeletal size, such as height at withers and body length, are hardly influenced by condition. The skeletal measurements are, therefore, considered more suitable as size measurements. On this basis, body size showed a fairly high correlation with fat production. The association between body capacity and fat production was not as high as between fat production and some of the other measurements.

Multiple correlation analyses. Cross-section areas of chest and paunch, body length (withers to pinbones), length from withers to hips, height at withers, and body weight were used in various multiple correlation analyses (1) with production as the dependent variable. The multiple correlation coefficients were compared with simple correlations between fat production and individual measurements, to determine whether such combinations of measurements were more closely associated with production than were single measurements. The results are presented in Table 3.

The standard partial regression coefficients of production on height at withers were all positive and significantly different from zero. When cross-section area of chest or body length was added to height at withers the multiple correlation coefficients only slightly exceeded the simple correlation between production and height at withers alone. The results of the analysis involving height at withers and body weight were interesting in that, within this system, the standard partial regression coefficient of production on weight was essentially zero. This indicated that the correlation found between weight and production (0.22) can be attributed mainly to the association between weight and height at withers.

The multiple correlation coefficient between height at withers, body length,

TABLE 3

Relationships between butterfat production *(Y)* and certain body measurements

Area chest (X_1)		Area paunch (X_2)		Length withers to hips (X_3)		Height at withers (X_4)		Length withers to pins (X_5)		Body weight (X_6)		Multiple correlation coefficients	
$P_{Y1.234}$[a]	.14	....		....		$P_{Y4.123}$	.45**	....		....		$R_{Y.1234}$	.37**
....		$P_{Y2.134}$	.07	....		$P_{Y4.213}$	.32**	....		....		$R_{Y.2134}$	.36**
....		....		....		$P_{Y4.312}$	.26**	$P_{Y5.124}$	.12	....		$R_{Y.3456}$	.36**
....		....		....		$P_{Y4.312}$	.38**	....		$P_{Y6.124}$	−.05	$R_{Y.1346}$	.35**
....		$P_{Y2.145}$	−.03	....		$P_{Y4.125}$	.52**	$P_{Y5.124}$	−.05	....		$R_{Y.12345}$	.40**
$P_{Y1.23}$	−.01	$P_{Y2.13}$	.15	$P_{Y3.12}$	.22*	....		....		....		$R_{Y.123}$	.31**
$P_{Y1.23456}$	−.31*	$P_{Y2.13456}$	.22	$P_{Y3.12456}$	−.07	$P_{Y4.12356}$	.42**	$P_{Y5.12364}$	.12	....		$R_{Y.123456}$	.40**
r_{Yy} .18*		.23**		.28**		.35**		.31*		.22**			

* $P<0.05$.

** $P<0.01$.

[a] Standard partial regression (path) coefficient.

and cross-section area of paunch as independent variables and production as the dependent variable was slightly higher than any of the correlations, including only two independent variables.

The analysis involving the cross-section areas of chest and paunch and length from withers to hips was a further effort to determine the association between body capacity and fat production. The multiple correlation coefficient was only slightly larger than the correlation between production and calculated body capacity (0.31 vs. 0.28). The multiple correlation, however, was still smaller than the correlation between production and some individual measurements.

Two measurements of skeletal size, height at withers and body length (withers to pinbones), were used in addition to the three measurements representing body capacity. The resulting multiple correlation coefficient was 0.40 as compared to 0.31. The standard partial regression coefficients, however, indicated that height at withers accounted for most of the variation in production which was accounted for by this set of variables. Also, the correlation was not higher than the one involving height at withers, body length, and area of paunch.

Since correlations between different body measurements are shown to be high (3, 9, 10), it is not surprising to find that multiple correlation coefficients between fat production and combinations of measurements do not exceed the simple correlation coefficients to any considerable degree.

Internal measurements in relation to external measurements and fat production. The correlations between external and internal measurements were

TABLE 4

Simple and partial correlations between various external and internal measurements

Relationship	Correlation coefficient	Partial correlation coefficient[a]
Cross-section area of chest and heart weight	+0.43**	+0.04
Cross-section area of chest and lung weight	−0.03	−0.15
Depth of chest and depth of thoracic cavity	+0.69**	+0.63**
Depth of chest and heart weight	+0.57**	+0.38**
Depth of chest and lung weight	+0.32**	+0.40**
Width of chest and width of thoracic cavity	+0.37**	+0.17
Cross-section area of paunch and liver weight	+0.32**	+0.09
Cross-section area of paunch and stomach weight	+0.34**	+0.41**
Cross-section area of paunch and length small intestines	+0.16	+0.13
Cross-section area of paunch and length large intestines	+0.19*	+0.12
Cross-section area of paunch and length total intestines	+0.19*	+0.14
Depth of paunch and liver weight	+0.48**	+0.34**
Depth of paunch and stomach weight	+0.38**	+0.43**
Depth of paunch and length small intestines	+0.31**	+0.31**
Depth of paunch and length large intestines	+0.37**	+0.35**
Depth of paunch and length total intestines	+0.36**	+0.35**
Height at withers and heart weight	+0.50**	+0.34**
Height at withers and lung weight	+0.28**	+0.29**
Height at withers and length total intestines	+0.09	+0.02
Body length and heart weight	+0.49**	+0.32**[b]
Body length and lung weight	+0.21[b]	+0.20[b]
Body length and total intestines	+0.34**	+0.22[b]

[a] Empty body weight held constant.
[b] Outbred animals only.
* $P<0.05$.
** $P<0.01$.

calculated on a within sire, within age group basis and weighted for mating systems (O, I_1, I_2). The cows could be grouped into two age classes, 3 to 5 yr. and 5 yr. and older, without losing too many degrees of freedom. One hundred and forty-six animals were included with 97 in O, 30 in I_1, and 19 in I_2. There were 31 subgroups with daughters of 22 sires. The results are given in Table 4.

It is likely that the simple correlations between body parts are biased upward, since the sample contained animals of varying skeletal size and condition. Furthermore, empty body weight was found to be significantly correlated with all external measurements (0.43 to 0.86), with depth and width of thoracic cavity, and with heart weight and liver weight. The correlations with lung weight, stomach weight, and length of intestines were small and not significantly different from zero. Since empty body weight is influenced by size and condition, an attempt was made to minimize the effect of size and condition by holding empty body weight constant. This was done by calculating partial correlations with empty body weight held constant. These are shown in Table 4.

Depth of chest was the only one of the chest measurements studied which provided some indication of the size of the thoracic cavity, and which also was positively associated with heart and lung weight. The cross-section area of the chest showed no relationship to lung weight, whereas the highly significant correlation with heart weight was a consequence of the influence of body weight on both of the measurements. The same applied for the relationship between external width and width of thoracic cavity.

The data show that depth of paunch may be regarded as a fairly good indication of the size of some organs of the digestive tract, such as stomach, small, large, and total intestines, and liver. The correlations between depth of paunch and weight or length of these organs were practically independent of body weight. The correlations between cross-section area of paunch and the internal organs were mostly lower (0.16 to 0.34) than was found for depth of paunch.

Height at withers was significantly correlated with heart and lung weight, with partial correlations remaining highly significant. However, it was not correlated with length of intestines. Body length was significantly correlated with heart weight and length of intestines. The correlation with lung weight approached significance. These correlations refer to outbred animals only. There were significant differences between the correlation coefficients for the outbred group and the inbred groups when body length was involved. The correlations for the three groups (O, I_1, I_2), therefore, could not be combined into a common estimate and the results are given for the outbred group only.

The correlations between fat production and internal measurements were calculated within year, sire and age-group subclasses, and weighted for different systems of mating. The age-at-slaughter classes were 3 to 4, 4 to 5, and 5 yr. and older. First-lactation production records, adjusted to 2 ×, 305-day, mature equivalent were used. The analysis included 115 animals, 83 in the O group, 22 in I_1, and 10 in I_2. The number of subgroups was 44, and 19 sires were represented.

The only significant correlations were between production and length of large intestines (0.29) and length of total intestines (0.25). The correlations

between production and length of small intestines (0.22), stomach weight (0.20), liver weight (0.23), and depth of thoracic cavity (0.20) approached significance at the 5% level of probability. Heart and lung weight failed to show significant correlations with production.

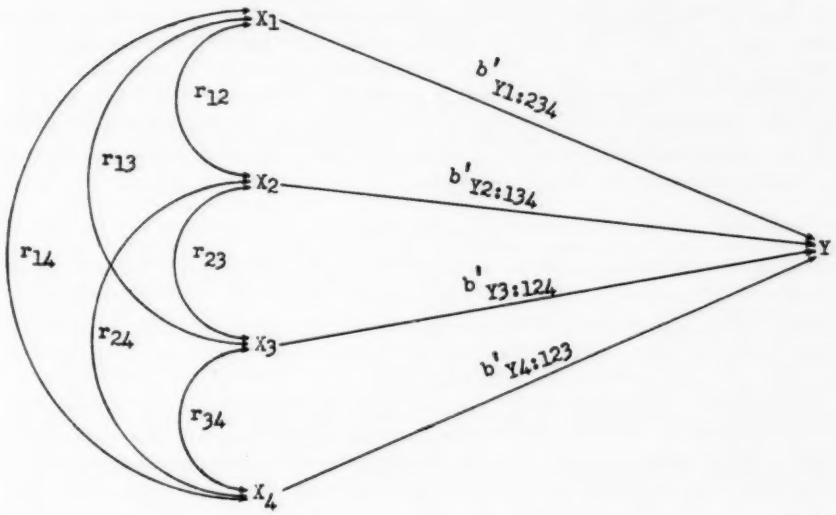
Previous work concerning internal anatomy usually did not include analyses of associations between size of internal organs and production (6, 7, 8). The present data indicate that slight associations exist between production and the size of certain organs of the digestive tract.

Path coefficient analysis—external body measurements and production. This study was made to determine if correlations between external measurements and fat production resulted from the fact that both were correlated with the size of internal organs. Path coefficients (4, 12) were used to partition the correlations to find the relative importance of direct and indirect paths of influence. All correlations between external and internal measurements, and between internal measurements and production, were examined for possible contributions to the correlations between external measurements and production. If the correlation between an external and an internal measurement or the correlation between the respective internal measurement and fat production was not significant, the association of the external measurement with production could not be attributed to its association with the internal measurement.

Among the correlations available only that between depth of paunch and production could be linked with interrelationships with internal measurements. These were length of intestines, liver weight, and stomach weight. All of these were significantly correlated with depth of paunch, and length of intestines was significantly correlated with production. The correlations between production and stomach weight, liver weight, and depth of paunch approached significance. The correlation between depth of paunch and production was separated into a direct path of influence from depth of paunch to production and into compound paths via the internal organs (Figure 1). The relative magnitude of the contribution of the direct path to the correlation between depth of paunch and butterfat production can be measured by the ratio of the respective path coefficient to the total correlation.

The within sire, year, and age-group phenotypic correlations weighted for mating systems (O, I_1, I_2) were used. The correlations between internal measurements and production were obtained with extremely small numbers of animals in the inbred groups. Some of the correlations turned out to be considerably different for outbred and inbred groups. Though these differences were not statistically significant, they were large enough to cause some concern. Therefore, a second analysis of associations between depth of paunch and production was made in which the same variables were included, but only the correlations for the outbred group were used. The results of these analyses are presented in Table 5.

The correlations between depth of paunch and fat production for the group including all animals (0.20) was smaller than it was for the outbred group (0.28). The path coefficients from depth of paunch to production ranked in the same order. Partitioning the correlation between depth of paunch and production into a direct path and the sum of the compound paths through the



$$r_{YX_1} = b'_{Y1:234} + r_{X_{12}} b'_{Y2:134} + r_{X_{13}} b'_{Y3:124} + r_{X_{14}} b'_{Y4:123}$$

$$\text{Relative contribution of the direct path} \qquad RC = \frac{b'_{Y1:234}}{r_{YX_1}}$$

FIG. 1. Path coefficient diagram indicating association between fat production (Y), depth of paunch (X_1), liver weight (X_2), stomach weight (X_3), and length of intestines (X_4).

association with liver weight, stomach weight, and length of intestines, gave different results for the two analyses. Considering the outbred animals only, 75% of the correlation between depth of paunch and production could be attributed to the direct influence of depth of paunch on production. The remainder was made up by the sum of the compound paths. On the other hand, the percentage of the correlation accounted for by the direct path was only 35% for the group with all animals included.

TABLE 5

Correlations and path coefficients between butterfat production and four external and internal measurements

Relationship	Correlations		Path coefficients	
	I[a]	II[b]	I[a]	II[b]
Production and depth of paunch	0.20	0.28	0.07	0.21
Production and liver weight	0.22	0.24	0.08	0.13
Production and stomach weight	0.20	0.18	0.08	0.01
Production and length of intestines	0.25	0.21	0.16	0.08

[a] I = all animals included.
[b] II = outbred animals only.

Some of the correlations used in obtaining these results did not quite reach significance at the 5% level of probability. Also, the path coefficients from the different measurements to production were not significantly different from zero. Therefore, care should be exercised in drawing conclusions from these results. It seems reasonable, however, to conclude that at least 25%, and possibly more, of the correlation between depth of paunch and fat production can be attributed to the association of depth of paunch with the size of internal organs.

CONCLUSIONS

The results of this analysis indicate that the external and internal body measurements studied account for only small portions of the variation in fat production. The measurements of the cross-section areas of chest and paunch do not seem to offer any advantages in predicting production over other common measurements which are more easily obtained.

A general supposition that external body conformation is an indicator of producing ability because of associations with internal organs and their interrelationships with production is not supported by the present findings. Among the correlations between external measurements and production analyzed in this study there is no evidence for interrelationships of this kind for measurements other than depth of paunch.

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PRODUCTION CHARACTERISTICS OF JERSEYS AND RED SINDHI–JERSEY CROSSBRED FEMALES [1]

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SUMMARY

Red Sindhis were crossed with Jerseys at three stations as an approach in developing dairy cattle adaptable to the southern United States. The production records of 180 cows in the crossbred groups of ¾ J, ⅝ J, F_1, F_2, and ¼ J were compared to their purebred Jersey stable-mates or contemporaries.

All the crossbred groups were lower in milk and fat yield, but tested slightly higher than their Jersey stable-mates. Level of milk and fat yield, length of lactation, persistency of milk yield, and feed efficiency decreased significantly as the proportion of Sindhi inheritance increased. The differences in production among the crosses at the three stations were almost directly proportional to the level of the Jersey stable-mates. Adjustment for length of lactation did not change the rank of the crossbred groups and was not the only cause of differences among breed groups.

Jerseys produced significantly more milk and fat than their maternal crossbred sisters sired by Sindhi and F_1 males, but were not significantly different from their purebred Jersey stable-mates.

Differences among crossbred groups were more important than sire differences within groups. Also, little improvement in the production, relative to their Jersey stable-mates, was shown in selected second lactations as compared to first lactation. Certain other characteristics exhibited by the crosses, i.e., temperament, rate of milking, udder characteristics, and eating habits, appeared to impair their usefulness as dairy animals.

The results of this study show no advantage of the Red Sindhi-Jersey crossbreds over purebred Jerseys in dairy characteristics under conditions prevailing at three locations.

The Red Sindhi is one of the numerous breeds of Zebu cattle found in India and Pakistan. Strains of this breed have been developed which have higher inherent milk-producing ability than is characteristic of Zebus as a whole. In 1946, two males and two females of the Red Sindhi breed were imported from the Allahabad Agricultural Institute, United Provinces, India. These Red Sindhi males and their sons were used in crossing with Jerseys, Holsteins, and Brown Swiss as an approach to the problem of development of dairy cattle adaptable to the southern United States.

The Red Sindhi–Jersey crossing was carried on at the Agricultural Research Center, Beltsville, Maryland; the Georgia Coastal Plain Experiment Station,

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Tifton, Georgia; and the Iberia Livestock Experiment Station, Jeanerette, Louisiana. Studies of growth, external body dimensions and surface area, gestation length, birth weight, age of first calving, and internal anatomical characteristics of these crosses have been reported previously (6, 7, 16). This paper deals with the milk and fat production, per cent fat, persistency of milk yield, and feed efficiency of the various combinations as compared to their purebred Jersey stable-mates or contemporaries at the three stations.

METHODS

Breeding plan. The breeding plan was to produce all combinations between the Jersey and Red Sindhi, by eighths, to determine the approximate fraction of inheritance from each desired for optimum productivity. The present study includes purebred Jerseys and ¾, ⅝, ½ (first generation), ½(second generation), and ¼ Jersey crosses made at one or more of the three stations. The number of animals with production information in other groups was too small for consideration. The stations will be referred to as Tifton, Jeanerette, and Beltsville, and the breed groups as Jersey, ¾ J, ⅝ J, F_1, F_2, and ¼ J, respectively.

Four purebred Red Sindhi bulls were mated to representative Jerseys in the Jeanerette and Beltsville station herds to produce the F_1 crosses. The ¾ J groups at Jeanerette and Tifton resulted from mating F_1 crossbred males to Jersey females, but the ¾ J group at Beltsville included reciprocals. There were six F_1 crossbred and three Jersey bulls used to produce these crosses. The F_1 crossbred bulls were sons of Jersey cows of above average production in the Beltsville herd and three of the Sindhi bulls. They were grouped in pairs and one pair used exclusively within a station. The ⅝ J crosses were produced by mating one of the F_1 crossbred males to ¾ J females. The F_2 group resulted from inter se mating two F_1 males with F_1 females. The four Sindhi males were mated to F_1 females to produce the ¼ J crosses.

Matings were made to keep inbreeding to a minimum. All crosses had less than 3% inbreeding except one of the ¼ J crosses at Jeanerette which was 25% inbred and the five ⅝ J crosses at Tifton which were all approximately 3% inbred.

Feeding and management. The within station feeding and management were similar for the crosses and the Jersey stable-mates. All cows were machine-milked twice daily except at Beltsville for the period December, 1949, to September, 1951, when 3× hand milking was practiced. At Beltsville all cows were barn-fed individually a standard ration based on recommendations for maintenance and production throughout all lactations. Refused feed was recorded, making it possible to calculate the total therms of estimated net energy (ENE) consumed. At Tifton and Jeanerette, concentrates were fed according to production. Hay and silage were fed ad libitum and pasture used in season. For the first 5 yr. all crosses were given an opportunity to complete at least two records, but in the last 4 yr. culling based on first-lactation production was practiced.

Statistical analyses of the data. The crossbreds were compared to purebred Jersey contemporaries or stable-mates. Some of the crosses at Beltsville did not have Jersey stable-mates; therefore, they were excluded from the study. The contemporaries were first-lactation Jerseys calving in the same year and season as the crossbreds. The stable-mates were all Jerseys calving in the same year and season. Essentially, these Jersey groups were unselected for production, since culling was based on disease, injury, or age.

The 3× records at Beltsville were adjusted to a 2×, 305-day basis with correction factors developed from Beltsville herd data. DHIA age-conversion factors were used to adjust the individual Jersey records to a mature equivalent (M.E.) basis. Records of both Jerseys and crossbreds were grouped into seasons by dates of calving (April-September or October-March). The year-season Jersey means were adjusted to the age of calving for each crossbred in the corresponding year-season and the deviation for each crossbred from the Jersey stable-mate average determined. The number of Jerseys was small in some year-season groups; therefore, the observed difference between the crossbred record and the year-season age adjusted record was weighted by the factor $\frac{n}{n+1}$. This procedure was considered more satisfactory than adjusting the crossbred record to a M.E. basis, since there was no way of estimating the reliability of Jersey age factors for use on the crossbred records.

To provide tests of significance and to evaluate the importance of some possible sources of variation of the weighted differences of all first crossbred records from their Jersey stable-mate averages, two least-squares analyses were completed. The following mathematical model was used to describe the first analysis and constants fitted:

$$y_{ijkl} = \mu + s_i + b_j + t_k + (sb)_{ij} + (st)_{ik} + (bt)_{jk} + c(A_{ijkl} - \overline{A}) + e_{ijkl}$$

Where: y_{ijkl} = the weighted difference between the l*th* crossbred record (milk or fat yield or fat per cent) started in the k*th* season in the j*th* breed group and at the i*th* station and the corresponding Jersey year-season averages.

c = the partial regression of the dependent variable (milk yield, fat yield, or fat per cent) on the age at freshening in months.

All breed groups were not present at all stations; therefore, only the three common breed groups at Beltsville and Jeanerette were considered in evaluating the $(sb)_{ij}$ interaction effects. In the second analysis the model was extended to remove statistically, by linear and quadratic regression, the effect of length of lactation.

Sixty-nine sets of maternal half-sisters were available at all three stations where at least one was a crossbred (F_1 or ¾ J) and at least one was a purebred Jersey daughter. In Analysis No. 1, constants were fitted for the three groups on a within dam or maternal half-sister set by use of the following mathematical model:

$$y_{ijkl} = \mu + s_i + d_{ij} + b_k + (sb)_{ik} + e_{ijkl}$$

Where: y_{ijkl} = the weighted difference of the 1st record of the l*th* daughter in the k*th* breed group out of the j*th* dam in the i*th* station from the stable-mate average. The differences for the purebred Jersey daughters were adjusted to the average age at calving for the crossbred daughters in each maternal half-sister set separately prior to the analysis. (First records only were used for the Jersey maternal half-sisters.)

In Analysis No. 2 the same constants were fitted as in Analysis No. 1 and, in addition, a linear partial regression was fitted for length of lactation.

The differences for the purebred Jersey daughters were adjusted to the average age at calving for the crossbred daughters.

Milk yields at 30-day intervals were available at Beltsville and Jeanerette for all crossbreds and contemporary Jerseys. These were used to calculate persistency indices for the various groups in the following manner:

$$\text{per cent P} = \left[\frac{2s(x > \bar{x}) - n(\bar{x})}{sx} \right] 100$$

Where: $s(x > \bar{x})$ = sum of yields for 30-day periods greater than the mean 30-day yield for the lactation.

2 = constant.

n = number periods when 30-day yield exceeded the mean for the lactation.

$\bar{x}$ = average yield of all 30-day periods.

sx = total of all 30-day yields.

RESULTS

Table 1 shows the average production of the crossbred groups, their Jersey stable-mates, and the weighted differences. The average for the crossbreds is the simple average of the actual records. The Jersey stable-mate average is the average of the year-season Jersey means adjusted to the age of the individual crosses. The weighted differences are the simple averages of the weighted deviations of each crossbred record from its age-adjusted Jersey stable-mate average. A total of 431 records of 228 Jersey stable-mates was used in determining the year-season averages. Of these there were 47 Jersey cows with 64 records at Beltsville, 72 cows with 162 records at Jeanerette, and 109 cows with 306 records at Tifton.

Except for the highly selected F_1 crosses which made third and fourth lactations at Jeanerette, the crossbreds produced less milk and fat than their Jersey stable-mates, but the weighted differences for per cent fat were variable. The fat test of the crosses was higher than the Jersey stable-mates in first lactation at Beltsville and the same was true for F_1 and ¼ J at Jeanerette. The ¾ J first lactation average for fat test at Jeanerette and Tifton was less than for the Jerseys. In later lactations the differences for all groups were negative except for the ¼ J at Jeanerette.

TABLE 1

Average milk and fat production of Sindhi-Jersey crosses for various lactations, the simple averages of the Jersey stable-mates, and the average of the weighted differences between the crossbred record and the year-season average for the purebred Jerseys

Breed group	Crossbred						Jersey stable-mate average [a]		Average of weighted differences [b]		
	Lactation No.	No. animal	Length of lactation	Age of calving (mo.)	Average Milk (10 lb.)	Average Fat (lb.)	Milk (10 lb.)	Fat (lb.)	Milk (10 lb.)	Fat (lb.)	Fat (%)
					Beltsville						
¾ J	1	10	305	26	722	429	792	457	− 58	− 22	.12
	2	3	305	41	745	427	849	497	−101	− 53	−.26
F_1	1	37	288	26	593	338	808	432	−175	− 74	.24
	2	18	285	41	572	325	946	521	−306	−160	−.07
	3	10	277	55	628	365	1,009	540	−340	−156	−.33
¼ J	1	15	212	27	316	182	811	447	−423	−227	.10
	2	5	272	40	554	305	855	483	−278	−175	−.13
					Jeanerette						
¾ J	1	10	295	30	546	246	569	268	− 22	− 20	−.21
	2	7	298	43	569	267	623	295	− 48	− 25	−.09
	3	3	265	56	616	291	667	325	− 44	− 30	−.04
F_1	1	47	256	29	461	233	572	270	−104	− 35	.37
	2	34	275	41	534	266	647	307	−105	− 38	−.03
	3	17	299	55	755	374	652	306	94	62	−.06
	4	13	298	68	794	392	664	312	117	72	−.13
F_2	1	10	249	30	376	168	568	268	−175	− 91	−.08
	2	6	276	46	512	213	610	286	− 86	− 65	−.53
¼ J	1	13	205	29	255	129	537	250	−252	−108	.41
	2	6	254	43	455	229	623	296	−153	− 61	.28
					Tifton						
¾ J	1	19	260	26	555	282	622	333	− 64	− 49	−.19
	2	6	240	37	519	275	641	343	−117	− 64	.01
⅝ J	1	5	265	26	568	257	639	344	− 68	− 82	−.77

[a] Average of weighted subclass Jersey means adjusted to age of calving for crossbreds.
[b] Average of weighted differences, not the arithmetic difference because of frequency of number.

TABLE 2

Least-squares means for weighted differences between Sindhi-Jersey crossbred records and Jersey stable-mate records (first records of crosses only)

Classification	No. animal	Independent variables [a]		Analysis No. 1 [b]			Analysis No. 2 [b]	
		Age of calving *(mo.)*	Length of lactation *(days)*	Milk *(10 lb.)*	Fat *(lb.)*	Fat *(%)*	Milk *(10 lb.)*	Fat *(lb.)*
Stations								
Beltsville	62	26	272	−201	−107	−.01	−235	−125
Jeanerette	80	29	251	−108	− 55	−.09	−113	− 57
Tifton	24	26	261	−165	−106	−.23	− 75	− 61
Over-all (μ)	166	28	261	−158	− 89	−.11	−141	− 81
Breed groups								
¾ J	39	27	281	− 41	− 27	−.12	− 88	− 51
⅝ J	5	36	265	− 68	− 64	−.56	−124	− 94
F_1	84	27	270	−145	− 63	.25	−138	− 59
F_2	10	30	249	−200	−118	−.27	−145	− 90
¼ J	28	28	209	−336	−175	−.15	−210	−110
Seasons								
April-September	77	28	252	−128	− 79	−.25	− 90	− 60
October-March	89	27	268	−188	−100	.03	−192	−102
Station-breed group								
¾ J Beltsville	10	26	305	− 65	− 22	.22	−144	− 63
Jeanerette	10	30	295	− 10	−14	−.34	− 98	− 60
F_1 Beltsville	37	26	288	−169	− 69	.27	−235	−102
Jeanerette	47	29	256	−113	− 40	.35	−107	− 37
¼ J Beltsville	15	27	212	−416	−226	.08	−338	−187
Jeanerette	13	29	205	−249	−107	.34	−146	− 54
Regressions								
Age of calving				1.55	1.57	.030	3.16	2.37
Length of lactation							− .722	− .258
Length of lactation squared							.007	.003

[a] Simple averages.
[b] Means for weighted differences.

The least-squares means of the weighted differences for first records that were obtained in both Analysis No. 1 and Analysis No. 2 for μ, stations, breed groups, seasons, and the station by breed group subclasses are presented in Table 2. In addition, Table 2 gives the number of crossbreds and the simple averages for age at calving and length of lactation for each classification. The partial regression estimates for age at calving and length of lactation are given at the bottom of Table 2.

In all cases for milk and fat yields the least-squares means of the weighted differences show that the crossbreds produced less than did their Jersey stablemates. In general, the weighted differences in milk and fat yields were larger negatively as the proportion of Sindhi inheritance increased. Since the average length of lactation varied from one classification group to another, the least-squares means for milk and fat yields differ between Analysis No. 1 and Analysis No. 2. In the latter analysis the least-squares means are each adjusted to the average length of lactation (261 days).

Tests of significance among the least-squares means for the main effects, the interaction constants, the partial regressions, and for the over-all mean itself are given in the analysis of variance shown in Table 3. When no adjustment was made for variations in length of lactation (Analysis No. 1) the error terms for milk and fat were so large that only the differences for all crossbreds (μ)

TABLE 3

Analyses of variance of the weighted differences between the crossbred records and the year-season age adjusted average for purebred Jerseys [a]

Source of variation	d.f.	Mean squares		
		Milk	Fat	Fat (%)
	Analysis No. 1			
μ	1	1,470,222**	470,474**	.6540
Stations (S)	2	64,236	21,654	.1635
Crossbred groups (B)	4	263,050**	80,928**	.9663*
Season (T)	1	53,485	6,080	1.1139
SB [b]	2	27,382	22,303	.9244*
ST	2	10,640	532	.3624
BT	4	22,377	2,486	.2277
Regression on age	1	1,488	1,525	.5577
Error	150	34,178	9,178	.2980
	Analysis No. 2			
μ	1	1,131,553**	372,971**	.7837
Stations (S)	2	152,244**	38,055**	.1923
Crossbred groups (B)	4	37,902**	15,264**	1.0152*
Seasons (T)	1	151,991**	25,779**	1.0507
SB [b]	2	28,342	23,327**	.9668*
ST	2	4,346	998	.3690
BT	4	8,402	2,408	.2486
Regression on age	1	6,179	3,484	.5286
Regression on length				
Linear	1	13,332	1,696	.3065
Quadratic	1	176,278**	39,523**	.2707
Error	148	12,321	3,196	.3013

* $P < .05$; ** $P < .01$.

[a] Adjusted for unequal subclass frequencies by least-squares. The year-season purebred Jersey average for fat per cent was unadjusted for age.

[b] All breed groups were not present at all stations.

and crossbred groups were significant statistically. Because of the wide variability in length of lactation among the crossbreds, and the large influence of length on both milk and fat yields, the error mean squares for these two variables under Analysis No. 2 are much less than under Analysis No. 1. Adjusting for length of lactation on per cent fat made little difference in the results obtained.

Differences among breed groups, when adjustment is made statistically for variation in lactation length, still remain highly significant (Analysis No. 2, Table 3). Therefore, the variability in average lactation length among breed groups is not the only reason for breed group differences in milk and fat yield. Under the assumption that the differences which actually occurred in the average length of lactation among stations, breed groups, and season (Table 2) are population characteristics, the error mean squares obtained in Analysis No. 2 may be used to test the significance of differences among the least-squares means of Analysis No. 1. When tests of significance are made in this manner, differences among stations and seasons are also significant for milk yield; and station, seasons, and the station by breed group interaction effects are significant for fat yield. There is no indication that a station by season interaction exists for either milk or fat yield, but there is some suggestion that a breed by season interaction exists for milk when no adjustment is made for lactation length.

The station least-squares means (Table 2) show that the differences among crossbred records and the Jersey stable-mates were larger (negatively) at Beltsville and Tifton than at Jeanerette. Table 1 shows that the production level for both crossbreds and Jerseys was considerably higher at Beltsville, suggesting that the station differences are largely due to a correlation between the mean and variance rather than to greater adaptability of the crossbreds at Jeanerette. The variation in the differences between Beltsville and Jeanerette for the three common breed groups (the station × breed group interaction) is also probably due primarily to the correlation of the mean and variance.

Results of applying Duncan's Multiple Range Test to differences among stations, breed groups, and station × breed group interaction constants are shown in Table 4. The error mean squares obtained in Analysis No. 2 were used

TABLE 4

Results of applying Duncan's Multiple Range Test to differences among stations and breed groups

Classification	Analysis No. 1	Analysis No. 2
Stations		
Milk	(B,T) (T,J)[a]	(B) (J,T)
Fat	(B,T) (J)	(B) (J,T)
% Fat	(B,J,T)	
Breed groups		
Milk	(¾J,⅝J) (⅝J,F₁,F₂) (¼J)	(¾J,⅝J,F₁,F₂) (⅝J,F₂,¼J)
Fat	(¾J,⅝J) (⅝J,F₁) (⅝J,F₂) (¼J)	(¾J,⅝J,F₁,F₂) (⅝J,F₂,¼J)
% Fat	(F₁,¼J) (¾J,F₂,¼J) (¾J,⅝J,F₂)	

Any groups that do not appear together within the same parentheses differ significantly ($< .05$).

Any groups that appear within the same parentheses do not differ significantly.

[a] B, Beltsville; J, Jeanerette; T, Tifton.

TABLE 5

Least-squares means for weighted differences between maternal half-sister records and stable-mate averages (first records only)

Classification	No. animal	Independent variables: Age of calving (mo.)	Independent variables: Length of lactation (days)	Analysis No. 1: Milk (10 lb.)	Analysis No. 1: Fat (lb.)	Analysis No. 1: Fat (%)	Analysis No. 2: Milk (10 lb.)	Analysis No. 2: Fat (lb.)
Stations								
Beltsville	61	30	300	−140	− 61	.04	−166	− 75
Jeanerette	76	29	281	− 83	− 22	.03	− 49	− 16
Tifton	46	26	278	− 8	− 13	.04	5	0
Over-all (μ)	183	29	286	− 77	− 32	.04	− 70	− 30
Breed groups								
Jersey	101	30	298	− 21	− 7	.14	− 41	− 19
F_1	58	27	270	−110	− 43	.23	− 69	− 23
¾ J	24	27	275	− 99	− 46	−.25	− 99	− 48
Station-breed group								
Jersey Beltsville	33	33	305	− 18	− 7	.13	− 55	− 26
Jeanerette	38	30	304	− 2	− 15	.11	− 61	− 31
Tifton	30	26	284	− 43	1	.17	− 9	− 1
F_1 Beltsville	25	26	292	−167	− 74	.10	−179	− 80
Jeanerette	33	28	253	−123	− 31	.35	− 33	4
¾ J Beltsville	3	25	305	−234	−103	−.12	−263	−118
Jeanerette	5	30	287	−124	− 19	−.36	− 52	− 20
Tifton	16	26	266	− 61	− 17	−.28	17	− 6
Regression								
Length of lactation							2.119	1.099

Results of Duncan's Multiple Range Test:	Analysis No. 1	Analysis No. 2
Milk	(J) (F₁,¾J)	(J,F₁,¾J)
Fat	(J) (F₁,¾J)	(J,F₁,¾J)
% Fat	(J,F₁) (¾J)	

to test the significance of differences among least-squares means obtained in both analyses. Duncan's Test for Analysis No. 1 shows that the milk production of the crosses, relative to their Jersey stable-mates, was less at Beltsville than at Jeanerette, although those at Tifton were not significantly different from the other stations. The Beltsville and Tifton crosses were different from Jeanerette in fat. There were no significant station differences for fat percentage.

The ¾ J group produced significantly more milk and fat than crosses with one-half or more Sindhi inheritance, but after consideration of length of lactation (Analysis No. 2) only the ¼ J differed significantly from the ¾ J.

The F_1 group had a higher fat test than any of the other groups except the ¼ J. The differences among the other groups were relatively minor.

Maternal half-sister analyses. The results of the analyses of data on 69 sets of maternal half-sisters are presented in Tables 5 and 6. Table 5 gives the least-squares means by stations, breed groups, station-breed group subclasses, and the tests of significance with Duncan's Test for breed groups. The analyses of variance are shown in Table 6.

TABLE 6

Analysis of variance in weighted differences for maternal sister data[a]

Source of variation	d.f.	Mean squares		
		Milk	Fat	% Fat
	Analysis No. 1			
Breed groups	2	154,147**	39,692**	.7772*
Station × breed groups	3	37,679	9,095	.2217
Dam × breed group within stations	71	26,837	6,405	.2035
Error	38	17,317	5,194	.2037
	Analysis No. 2			
Breed groups	2	28,486	4,546	.7749*
Station × breed groups	3	78,800**	21,670**	.2117
Regression on length	1	1,232,891**	332,360**	.0027
Error	108	12,320	2,961	.2054

* $P < .05$; ** $P < .01$.

[a] In each analysis constants were also fitted for stations and dams within station. Constants for the dam × breed group interaction within stations in Analysis No. 1 were not fitted.

In the analysis of variance, when length of lactation was held constant (Analysis No. 2), the differences between Jerseys and their crossbred maternal sisters were not significant for milk and fat yield, but were for fat test (Table 6). The station × breed group interaction approached significance in Analysis No. 1 for milk, but was significant in Analysis No. 2 for both milk and fat. There was no indication of an interaction for fat test in either analysis. The regressions of milk and fat yields on length of lactation were significant in Analysis No. 2.

Jersey offspring of Jersey cows produced significantly more milk and fat relative to their stable-mates than did their maternal sisters sired by Sindhi and F_1 males (Table 5). The Jerseys had a significantly higher fat test than

their sisters sired by F_1 males, but were slightly lower than their F_1 sisters. The differences among the groups of maternal sisters were larger at Beltsville than at Jeanerette or Tifton in both sets of least-squares means. The Jersey maternal sibs were not significantly different from their Jersey stable-mates in milk, fat, or test at any of the stations.

Per cent difference in milk and fat yields. Table 7 shows little improvement

TABLE 7

Weighted average per cent decrease of crossbred from Jersey stable-mates computed from Table 1

	1st Lactation			2nd Lactation			
Breed group	No. animal	Milk (%)	Fat (%)	No. animal	Milk (%)	Fat (%)	Stations
¾ J	39	− 8	−10	10	−10	−11	Beltsville, Jeanerette, Tifton
⅝ J	5	−11	−25				Tifton
F_1	84	−20	−15	52	−27	−25	Beltsville, Jeanerette
F_2	10	−32	−34	6	−16	−26	Jeanerette
¼ J	28	−50	−48	11	−31	−31	Beltsville, Jeanerette

in the production of the crosses relative to their Jersey stable-mates in second lactation in spite of the culling that occurred between first and second lactation in the crossbred groups. The differences in first lactation ranged from a low of 10 to 50% decrease from purebred Jerseys and from 10 to 31% in second lactation.

Comparison of Sindhi sire groups. The adjusted means of F_1 crossbred daughters of three Sindhi sires are shown in Table 8. There were large but

TABLE 8

Adjusted means[a] of F_1 crosses for weighted differences from purebred Jersey stable-mates by sire groups at Beltsville and Jeanerette (first records only)

Red Sindhi Sire No.	No.[b] animal	Analysis No. 1			Analysis No. 2		
		Milk (10 lb.)	Fat (lb.)	Fat (%)	Milk (10 lb.)	Fat (lb.)	Fat (%)
50	21	−193	−94	.12	−157	−75	.14
51	50	−127	−51	.37	−129	−47	.37
52	13	−131	−75	.04	−141	−81	.04

[a] Daughter averages adjusted for the effects of station, season, station × season, and breed group × station interactions.

[b] Station distribution: Sire No. 50—17 Beltsville, 4 Jeanerette; Sire No. 51—7 Beltsville, 43 Jeanerette; Sire No. 52—13 Beltsville.

nonsignificant differences in the milk and fat yields and fat test of their daughters' first records. Holding length of lactation constant (Analysis No. 2) appreciably reduced differences in milk among the sires.

Persistency. In general, first-lactation persistency of milk yield was inversely related to the proportion of Sindhi inheritance at both Beltsville and Jeanerette (Table 9). The lower means represent high persistency. At both

TABLE 9

First-lactation persistency of milk production for Sindhi-Jersey crosses and Jersey contemporaries

Breed group	Beltsville		Jeanerette	
	No. animal	Mean	No. animal	Mean
Jersey	20	18.9 ± 1.38	35	23.2 ± 1.64
¾ J	10	14.6 ± 5.47	11	29.4 ± 5.86
F_1	37	26.4 ± 5.34	44	40.8 ± 6.82
F_2			10	51.5 ± 14.65
¼ J	15	78.7 ± 14.21	13	72.7 ± 16.68

Results of Duncan's Multiple Range Test: Beltsville (J, ¾ J, F_1) (¼ J); Jeanerette (J, ¾ J, F_1, F_2) (F_1, F_2, ¼ J).

stations the Jerseys and ¾ J had significantly higher persistency than the ¼ J, but were not different from the F_1. The F_1 and F_2 were not different from the ¼ J at Jeanerette.

Feed efficiency. Feed efficiency expressed as pounds of FCM produced per therm of estimated net energy consumed decreased with increasing Sindhi inheritance (Table 10). The total energy consumed from roughage per unit

TABLE 10

Feed efficiency of Sindhi-Jersey crosses and Jersey contemporaries at Beltsville (first lactation only)

Item	Jersey	¾ J	F_1	¼ J
No. of animals	18	11	25	11
Feed efficiency *(lb.FCM/therms ENE consumed)*	1.80	1.78	1.62	1.45
Coefficient of variation *(%)*	10.9	8.1	11.3	30.4
Body weight *(lb.)*[a]	912	884	894	787
Body weight gain *(lb.)*[b]	108	125	131	85
FCM *(10 lb.)*	938	919	698	399
Therms of roughage consumed/ 100 lb. body weight/day	1.05	1.08	.82	.78
Total therms consumed/100 lb./day	1.87	1.92	1.62	1.55
Results of Duncan's Multiple Range Test:				
Feed efficiency (lb. FCM/therms)			(J,¾J) (F_1,¼J)	
Therms consumed in roughage/100 lb. weight			(J,¾J) (F_1,¼J)	
Therms consumed/100 lb. weight/day			(J,¾J) (F_1,¼J)	

[a] Average of monthly weights during lactation.
[b] Difference between first month post-partum and last month of lactation.

of weight per day was similar in the Jerseys and ¾ J, but considerably greater than the F_1 and ¼ J groups. The differences in body weight gain among the Jerseys, ¾ J, and F_1 groups were small and all were greater than the ¼ J group. The Jerseys and ¾ J groups were significantly higher in feed efficiency, energy consumed, and roughage consumption than the F_1 and ¼ J groups.

DISCUSSION

The Sindhi-Jersey crosses were on the average inferior to their Jersey stable-mates under the temperate climatic conditions of Beltsville as well as the

warmer conditions at the two other stations. All crossbred groups were also highly variable in production. The first-lactation milk yields of the ¾ J ranged from 3,000–9,000 lb.; the F_1, 100–8,600 lb.; the F_2, 1,000–6,200 lb., and the ¼ J, 200–6,880 lb. In all combinations the distribution in milk yields was continuous and did not contain groups of extremes. Because of the large differences in the means and the distribution in milk yields, a large percentage of the crosses were unsatisfactory producers.

Undesirable temperament was partially responsible for the lower production of the crosses, especially in the F_1, F_2, and ¼ J crosses.

The inverse relation of lactation length and Sindhi inheritance is in agreement with that reported by Sen *et al.* (13) for Red Sindhi–Ayrshire crosses. Adjustment to a common length of lactation penalized the ¾ and ⅝ J, but helped the F_1, F_2, and ¼ J groups. Length of lactation was, therefore, an important factor in the performance of crosses but was not the sole cause of their lower yields.

Others (14, 15, 17) have reported that high percentages of Sindhi delayed sexual maturity. Although there was some indication of this in the ¼ J (6), the mean calving age for all crossbred groups was similar to that of their Jersey contemporaries. The advanced age at Jeanerette was due to deliberate delay in age of breeding.

There is little indication that the relative production of the Jerseys and crosses changed in later lactations. Thirteen of the 17 lowest-producing F_1 crosses at Jeanerette averaged 1,780 lb. of milk in first lactation and 3,460 lb. in second lactation. Even with some selection for production, the ¾ J and F_1 produced less in relation to their Jersey stable-mates in second than in first lactation. The highly selected group of F_1 crosses completing third and fourth lactations at Jeanerette exceeded the average of the Jersey stable-mates, but were below the average of the high 40% of the Jerseys. Some improvement was shown in the second-lactation production, relative to the Jersey stable-mates, of the F_2 and ¼ J groups, but this resulted primarily from removal of low-producers.

The inferiority of the crosses in first lactation was apparently not a maturity factor, as the increases in milk yield from first to second and second to third lactations were not any more than for the Jerseys. There is some indication the crosses increased less with age.

Generally, the first lactation fat test of the crosses was higher than for the Jersey stable-mates, but the pattern was in most cases reversed in second lactation. The lower test of the crossbreds in second lactation was probably a result of sampling, as those crossbreds completing second lactations tested below their respective group averages in first lactation. Lack of consideration of age for the Jersey stable-mates may have also been partially responsible for the positive values of the crosses in first lactation. Sire effects were additional confounding factors in the differences for the ¾ and ⅝ J groups. The significant station by breed group interaction for fat test could have resulted from differences between the purebred Jerseys at the two stations involved, Beltsville and Jeanerette.

Stonaker *et al.* (14) reported that first-lactation Jersey–Red Sindhi crosses tested higher than contemporary Red Sindhis; whereas, the present study shows the crosses tended to test higher than Jerseys. The true effects of crossing the two breeds on fat test can not be discerned from these studies, but both indicate that crosses test higher than either parent.

Some of the crossbred groups produced more relative to Jerseys during the summer months than in winter, but the level of production was still less than that of the Jerseys milking at the same time. The performance of the crosses during the hotter season might be explained on the basis of physiological studies conducted under hot conditions in psychrometric chambers. Under the conditions of high temperature and humidity, the responses of the ¾ J crosses were very similar to those of contemporary Jerseys, but the responses of the F_1 and ¼ J were significantly less than those of Jerseys (10). As compared to the Jersey, the average crossbred maintained a lower body temperature (10, 12); had a higher respiratory rate as a lactating animal (5, 9); showed a greater reduction in respiratory tidal volume (9); had lower metabolic heat production (2); and had rates of surface evaporation equal to or exceeding the Jersey (5, 8).

The differences between the Jersey maternal sisters and their purebred stable-mates were generally small and unimportant, which shows that the major portion of the dams of the crossbreds were representative of the Jerseys in the three herds. Therefore, it should be reasonable to assume that the decrease in production with increasing Sindhi resulted from breed or sire differences and not from the variation in the transmitting ability of the Jersey dams.

Although there was variation in the average production of the F_1 offspring by the three Sindhi sires used most extensively, the best of these groups produced much less than their Jersey stable-mates. The performance of the poorest of these F_1 groups, however, was above that of the best group of ¼ J paternal sibs. The lack of improvement through crossing would raise a question as to the quality of the Sindhi sires used. S-50 and S-51 were imported and S-52 was a son of S-51. Both S-50 and S-51 were out of dams with records well above the Allahabad herd average and that generally given for the breed in India. It is reasonable, therefore, to expect these animals to have transmitted at least breed average production.

The feed efficiency means reflect the high correlation between feed efficiency and FCM yield reported for dairy cattle (1). In general, energy intake was inversely related to the amount of Sindhi in both roughage and grain, but the decrease was greater for roughage. The lowered roughage consumption of the crosses may be a reflection of the smaller stomach and intestine capacity of the Sindhi crosses reported by Swett *et al.* (16).

Certain other characteristics exhibited by the crosses impressed the authors as factors which might affect their usefulness as dairy animals. The crosses were slow or piece-meal eaters, i.e., they consumed their feed in the barn or on pasture at a much slower rate than Jerseys or Holsteins. This was most evident in the ¼ J groups. Rate of milking appeared slower and directly related to the proportion of Sindhi. The crosses with 50% or more Sindhi usually had

larger fore than rear quarters and seemed to milk slightly more from the fore quarters. With advancing age, the fore-teats grew to such dimensions that they hampered the use of the milking machine and discouraged nursing by the calves. The crosses and the pure Sindhis carried more flesh at all times than Jerseys, which indicated they had a stronger tendency to maintain good fleshing than to milk. Also, the crossbred calves with 50% or more Sindhi were troublesome to rear, in that they were finicky eaters and slow in learning to drink from a pail.

Studies of summer weather patterns occurring in the South (3) showed the Jeanerette area to be among the most critical areas in the United States for the performance of dairy cattle. At Jeanerette the performance of the crosses relative to Jerseys was better than at the other stations. Nevertheless, the number of discards would have been high even under these conditions. The ¾ J were the best producers among the crosses, but their heat adaptation showed no material advantage over the Jerseys. The F_1's showed a significant improvement in heat resistance, but the productive performance of the top one-third of this group was not at a level that encouraged attempts to fix the greater adaptability characteristics. The disadvantages of the crosses in other traits normally desired in United States dairy cattle offered further discouragement. Although the general results were not encouraging, the performance of the crosses at Jeanerette would indicate that the use of European × Zebu crosses might prove beneficial under actual tropical conditions where heat-tolerant cattle now exist but European breeds have not been satisfactory.

From the results of this study and the knowledge that variation in adaptability exists among the European breeds, it would appear that research on systems of breeding other than Red Sindhi–Jersey crossing would offer greater promise for developing adaptability to conditions in the southern United States.

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EFFECTS OF LONG-TIME FEEDING OF CHLORTETRACYCLINE TO DAIRY CATTLE [1, 2]

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SUMMARY

Forty dairy heifers paired by age and breed were divided into two groups, with one of each pair receiving 240 mg. chlortetracycline for a minimum of 15 mo. before parturition and subsequently except when dry. The mean weight for the antibiotic-fed group exceeded that of the controls at 12 mo. of age by 38 lb., at 18 mo. of age by 39 lb., and at ten days after calving by 44 lb. Age at first breeding was less for the chlortetracycline group, but since this group required more services per conception the ages at first calving for the two groups were similar. Calves from antibiotic-fed heifers were slightly larger at birth, but response of the calves to chlortetracycline feeding appeared to be independent of treatment of the dam. A total of 30 and 32 records were made by treated and control animals, respectively, in three lactations. Mean total pounds milk and fat during the first lactation (adjusted to 2×, 305-day, M.E.) were 11,088 and 435 for treated cows and 10,615 and 399 for controls, respectively. Only the difference in fat yields was significant statistically ($P < .05$). Average adjusted milk and fat productions, in pounds, for all lactations were 10,531 and 409 for treated animals and 10,869 and 407 for controls. These differences were not significant (at $P = .05$). Although culling procedures limited comparisons, there apparently was little difference between groups in reasons for leaving the herd.

The effects of feeding antibiotics to dairy calves have been investigated extensively in recent years. Lassiter (9), in a review, reported that in most experiments chlortetracycline feeding improved general health and increased weight gains of dairy calves. This author concluded that there is little economic advantage in including antibiotics in the diet of dairy calves beyond 16 wk. of age. Also, it has been demonstrated that the most pronounced effects of dietary chlortetracycline are manifested during the first 6 mo. of life (3, 6, 7, 15). Several studies have indicated that chlortetracycline feeding to lactating dairy cows has no appreciable effect on milk yield or composition (1, 4, 12, 14, 16, 17). Lassiter and Brown (10), however, conducted a field study involving approximately 1,000 cows in 18 herds. The cows in each herd were divided into two groups; one group received chlortetracycline at the rate of 0.1 mg. per pound of body weight daily, and the other group served as the control. The antibiotic-fed cows in this experiment produced 0.82 lb. more milk per cow daily than the controls. Feeding chlortetracycline at levels of 700 (12) and 390 (13) mg. per animal and 0.1 mg. per pound body weight (5, 18) daily to lactating cows did not result in the appearance of detectable amounts of the antibiotic in the milk. Shor *et al.* (18), on the other hand, found small amounts

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of the antibiotic in the milk when chlortetracycline was fed at a level of 500 mg. per animal daily.

There has been considerable interest recently in feeding chlortetracycline to mature dairy cattle to increase resistance to certain bacterial infections. Bold *et al.* (2), however, found no apparent increase in resistance to mastitis, foot rot, or other bacterial disturbances as a result of chlortetracycline feeding, although the clinical disease challenge to these ailments was considered low. Furthermore, Ross *et al.* (14) reported no effect upon mastitis or foot rot as a result of chlortetracycline feeding.

The present experiment was undertaken to determine the effect of long-time feeding of chlortetracycline on the performance of dairy cattle. Portions of the data have been summarized previously (3, 7).

EXPERIMENTAL PROCEDURES

Forty dairy heifers in the Iowa State University herd were divided into two comparable groups on the basis of age and breed. Most of the treated animals were fed chlortetracycline from four days of age, although the antibiotic was introduced into the diet of some animals at a later age. This was not considered in the analysis, however, since all animals in the experimental group were fed chlortetracycline at the rate of 240 mg. per animal per day, added to the concentrate mixture in two equal feedings daily, for at least 15 mo. prior to calving. Thereafter, chlortetracycline was fed at this level as long as the cows were lactating, but not during their dry periods. In other respects all of the animals were fed and managed uniformly throughout their lifetime in the herd. No effort was made to retain animals in the herd beyond the time normal culling procedures indicated their removal.

Recorded data included daily milk weights, monthly milk fat percentages, body weight changes, and birth weights of offspring from first parturition. The mean weights at four days of age of the heifers assigned to the experiment (Table 1) averaged 81 lb. for the chlortetracycline group and 79 lb. for the controls.

The effect of treatment (chlortetracycline vs. none) of the heifers on the performance of their calves from the first parturition was studied. Ten calves from treated dams and ten from the nontreated dams were divided into four relatively comparable groups. Because of differences in time of calving and sex of offspring by breeds, it was possible to use only 20 calves. Each calf received whole milk for 7 wk., a starter ad libitum up to 4 lb. per calf daily, and good-quality alfalfa hay free choice. Two groups, one from each group of dams, received chlortetracycline (80 mg. per animal daily) in the form of Aurofac D in the milk during the first 7 wk. and as Aurofac 2A in the starter thereafter. Management practices were similar for all calves.

RESULTS AND DISCUSSION

The weights of the heifers at various ages are shown in Table 1. At 12 mo. of age the mean weight for the chlortetracycline group exceeded that of the

TABLE 1

Body weight changes [a]

Chlortetracycline						Control					
Heifer breed and No.	Date of birth	Wt. at 4 days	Wt. at 12 mo.	Wt. at 18 mo.	Wt. at 10 days following calving	Heifer breed and No.	Date of birth	Wt. at 4 days	Wt. at 12 mo.	Wt. at 18 mo.	Wt. at 10 days following calving
		(lb.)	*(lb.)*	*(lb.)*	*(lb.)*			*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
A 3520	2-4-51	70	619	796	988	A 3452	9-4-50	70	532	705	990
A 3533	3-6-51	77	635	840	1,049	A 3521	2-12-51	73	557	693	904
G 3525	2-18-51	79	510	678	790	G 3480	11-12-50	58	422	611	786
G 3530	2-28-51	79	532	679	916	G 3532	3-4-51	65	383	568	745
G 3544	4-10-51	66	521	715	783	G 3577	6-20-51	67	518	751	856
G 3587	7-21-51	64	508	721	943	G 3586	7-20-51	58	495	741	877
J 3499	12-22-50	63	545	692	844	J 3470	10-19-50	55	577	642	761
J 3542	4-5-51	64	499	709	974	J 3504	12-30-50	56	419	566	751
H 3535	3-23-51	95	598	821	1,022	H 3541	4-2-51	102	609	853	1,044
H 3552	4-22-51	88	631	873	985	H 3550	4-21-51	101	620	881	1,136
H 3557	5-3-51	93	650	864	1,152	H 3554	4-27-51	109	686	960	1,132
H 3564	5-14-51	105	592	836	1,111	H 3565	5-21-51	102	621	877	1,065
H 3576	6-19-51	92	628	914	1,074	H 3578	6-27-51	95	579	880	968
H 3606	9-21-51	87	611	854	1,138	H 3588	7-22-51	87	547	750	939
H 3608	9-23-51	84	559	770	1,029	H 3591	8-5-51	83	520	768	957
H 3609	9-24-51	92	623	845	1,025	H 3593	8-13-51	84	633	893	972
H 3616	10-22-51	85	647	874	1,084	H 3604	9-19-51	91	684	936	1,085
B 3562	5-11-51	92	661	900	1,079	B 3553	4-25-51	87	515	706	—[c]
H 3622	11-11-51	101	675	940	—[b]	H 3594	8-13-51	70	457	600	—[d]
J 3560	5-4-51	50	399	562	689	J 3582	7-15-51	68	500	724	—[e]
Total		1,626	11,643	15,883	18,675			1,581	10,874	15,105	15,968
Mean		81	582	794	983			79	544	755	939

[a] All heifers received 240 mg. chlortetracycline daily (120 mg/feeding) at least 15 mo. prior to calving.
[b] Aborted.
[c] Nonbreeder.
[d] Sold—Brucellosis reactor.
[e] Sold before parturition.

TABLE 2

Analysis of variance of weights at 12 mo. of age

Source of variation	Degrees of freedom	Mean square	F value
Breeds	4	31,030	10.06**
Treatments	1	14,784	4.79*
Breeds × treatments	4	3,619	1.17
Experimental error	30	3,085	.....

* $P < .05$.
** $P < .01$.

controls by 38 lb. Analysis of variance of these data (Table 2) indicated a statistically significant difference among breeds ($P < .01$) and between treatments ($P < .05$). This greater increase in body weight, from nearly identical birth weights, by chlortetracycline-fed heifers as compared to the controls is in agreement with previously reported work (6, 8, 14). A difference in body weights between treatments and among breeds was maintained through 18 mo. of age. The treatment differences were proportionately smaller than at 12 mo. of age and as shown in Table 3 were not statistically significant (at $P = .05$),

TABLE 3

Analysis of variance of weights at 18 mo. of age

Source of variation	Degrees of freedom	Mean square	F value
Breeds	4	69,056	11.79**
Treatments	1	15,132	2.58
Breeds × treatments	4	5,432	0.93
Experimental error	30	5,859	.....
Total	39	.....	.....

** $P < .01$.

although there was a significant difference ($P < .01$) among breeds, as was observed at 12 mo. The reason for the lack of added response to chlortetracycline feeding beyond 12 mo. of age probably can be explained by the rapid disappearance of the antibiotic from the rumen after prolonged feeding to older animals as reported by Lodge *et al.* (11). The fact that chlortetracycline apparently disappears from the rumen at a rapid rate at this age raises some question as to the role it plays in improving the health of older ruminants. Proper assessment depends upon whether this disappearance is due to absorption or to destruction (or alteration) in the rumen.

Weight and age differences between treatments at time of breeding are shown in Table 4. Mean body weight and age at first breeding for chlortetracycline-fed heifers and for controls, respectively, were 698 lb., 469 days, and 676 lb., 487 days. The treated heifers required an average of 2.2 services per conception, whereas the controls required 1.5, but numbers were not sufficient to permit valid conclusions as to the effect of chlortetracycline on the number of services per conception. The mean ages at conception were almost identical (505 days for the antibiotic group and 507 days for the controls). Body weights

TABLE 4

Reproductive efficiency (first conception)

Chlortetracycline						Control					
Heifer breed and No.	First breeding		Age at conception	Age at calving	Services per conception	Heifer breed and No.	First breeding		Age at conception	Age at calving	Services per conception
	Weight	Age					Weight	Age			
	(lb.)	*(days)*	*(days)*	*(days)*			*(lb.)*	*(days)*	*(days)*	*(days)*	
A 3520	763	465	465	668[a]	1	A 3452	674	508	530	816	2
A 3533	793	526	571	857	2	A 3521	654	494	494	759	1
G 3525	614	454	454	731	1	G 3480	624	564	564	849	1
G 3530	626	455	455	728	1	G 3532	516	480	480	755	1
G 3544	600	458	479	761	2	G 3577	615	447	447	724	1
G 3587	607	461	502	789	3	G 3586	644	456	549	825	3
J 3499	571	404	455	737	2	J 3470	540	452	452	726	1
J 3542	582	458	642	934	7	J 3504	569	548	548	826	1
H 3535	720	468	575	851	3	H 3541	720	463	463	738	1
H 3552	689	448	448	713	1	H 3550	710	450	450	729	1
H 3557	686	442	510	791	2	H 3554	806	477	538	809	2
H 3564	887	581	581	865	1	H 3565	714	458	477	758	2
H 3576	809	482	482	762	1	H 3578	754	470	470	735	1
H 3606	703	443	544	817	4	H 3588	714	497	497	773	1
H 3608	708	497	536	815	3	H 3591	707	491	510	787	2
H 3609	717	447	470	742	2	H 3593	808	493	493	768	1
H 3616	780	469	541	843	2	H 3604	808	456	493	765	3
B 3562	790	476	494	782	2	B 3553	697	532	.....		—[c]
H 3622	790	446	470	—[b]	2	H 3594	608	558	563		1[d]
J 3560	524	496	496	792	1	J 3582	636	452	583		3[e]
Total	13,959	9,376	10,170	14,978	43		13,518	9,746	9,601	13,142	29
Mean	698	469	509	788	2.2		676	487	505	773	1.5[f]

[a] Aborted 203 days—milked.
[b] Aborted—not milked.
[c] Nonbreeder.
[d] Sold—Brucellosis reactor.
[e] Sold before parturition.
[f] Does not include animals that failed to conceive.

ten days following parturition (Table 1) averaged 983 lb. for the antibiotic-fed heifers and 939 lb. for the controls, or approximately the same weight differences as observed at 12 and 18 mo. of age. The larger size apparently resulted in earlier sexual development in the antibiotic group, although a somewhat lower conception rate was observed.

The effects of chlortetracycline on the birth weight of offspring and on the response of the first parturition calves to this antibiotic are shown in Table 5.

TABLE 5

Mean body weight changes of the various groups of calves

Breed and sex distribution	Dam group	Calf group	Wt. at 4 days	Wt. gain to (days): 32	60	88	116
				(lb.)			
1 J-F, 1 G-F, 3 H-M	Chlortetracycline	Chlortetracycline	78	14	52	102	148
1 J-F, 1 G-F, 3 H-M	Chlortetracycline	Control	79	8	44	78	125
1 G-F, 2 H-F, 2 H-M	Control	Chlortetracycline	74	17	57	96	149
1 G-F, 2 H-F, 2 H-M	Control	Control	74	6	35	71	112

Calves from both treated and nontreated cows responded similarly to chlortetracycline feeding during the period from four to 116 days of age by gaining more than their controls that did not receive the antibiotic. The controls from antibiotic-fed dams, however, gained more than the controls from control dams. Unfortunately, numbers were too few to ascertain conclusively whether this represents a true difference.

Analyses of milk production data were carried out on first lactation separately and on all lactations through the third. Sufficient records were available to justify analysis of first lactation data alone; however, the numbers of neither second nor third lactations were sufficient to justify separate analysis. Thus, the first, second, and third were pooled to give a combined analysis of all data. Production data beyond the third lactation were deemed too limited for inclusion. Table 6 presents the mean yields [2×, 305-day, M.E. (8)] of milk and

TABLE 6

Average production (2×, 305-day, M.E.)

Lactation	Fat		Milk	
	Chlortetracycline	Control	Chlortetracycline	Control
1st	435 (17)[a]	399 (17)	11,088 (17)	10,615 (17)
2nd	377 (10)	408 (11)	10,317 (10)	11,085 (11)
3rd	362 (3)	436 (4)	8,088 (3)	11,354 (4)
All	409 (30)	407 (32)	10,531 (30)	10,869 (32)

[a] The numbers in parentheses give the numbers of animals in each subclass.

fat by lactations for the chlortetracycline and control groups. The numbers in parentheses indicate the number of cows in each lactation group. First-lactation fat production was 36 lb. higher for the chlortetracycline-fed cows than for the controls. In the second lactation, however, fat production of the con-

trols was 31 lb. higher. The mean fat productions of the two groups for all lactations were nearly the same. Milk yield tended to follow the same general pattern; however, the production in all lactations was slightly higher for the control animals.

The analysis of first lactation data is presented in Table 7. Sources of

TABLE 7

First lactation mean squares

		Actual		2×, 305-day, M.E.	
	d.f.	Fat	Milk	Fat	Milk
Treatments	1	7,650*	1,533,613	10,944	1,907,376
Breeds	3	9,561*	55,255,105**	21,541**	101,665,050**
T × B	3	3,677	111,657	5,552	195,552
E	26	1,819	1,593,266	2,748	2,502,518

* $P < .05$.
** $P < .01$.

variation due to treatments, breed, and their interaction were separated, showing that the only significant treatment effect ($P < .05$) was for actual fat production in the first lactation; however, the treatment effect on 2×, 305-day, M.E. fat production was significant at the 10% level. There was not a significant effect of chlortetracycline on milk production for either first-lactation data or all lactations combined in either actual or mature equivalent records. Even though the original design of the experiment insured that animals were paired by age within breed when they were allocated to the experiment, considerable age differences existed between the chlortetracycline and control groups in subsequent lactations. Adjustment for age differences was necessary before those data could be pooled. Accordingly, all data were converted [2×, 305-day, M.E. (8)] and an analysis similar to that in Table 7 was carried out for mature equivalent milk and fat production (Table 8). The effect of

TABLE 8

All lactations mean squares

		2×, 305-day, M.E.	
	d.f.	Fat	Milk
Treatments	1	53	1,764,210
Breeds	3	29,014**	147,332,913**
T × B	3	1,966	1,076,143
E	54	3,699	2,804,723

** $P < .01$.

chlortetracycline was negligible in the all-lactation data. In both Table 7 and Table 8, the differences between breeds are consistently apparent, as would be expected.

Since relatively small numbers of animals were involved, and because there was some question as to whether age-correction factors would be applicable, an analysis was carried out for both milk and fat production, using mature

equivalent records. This analysis considered sources of variation due to treatments, breeds, lactations, and their interactions. Lactation differences were not significant, indicating that the age-correction factors were at least roughly applicable to the present population.

Data collected in the Iowa State University herd from 1940 through 1958 indicate that some seasonal differences in production may occur. When records are classified by month of calving into two seasonal groups, lower production is encountered in cows that calve in the months of March through August, inclusive, than in those calving in the period of September through February. During the time when the data reported herein were collected, calving occurred in both of the above periods. Accordingly, the effect of season of calving on these records was considered. Analyses of first lactation and all lactations [2×, 305-day, M.E. (8)] considering the sources of variation due to treatments, breeds, seasons, and their interactions, indicated that seasonal differences per se were unimportant; however, there was a significant ($P < .01$) treatment-by-season interaction in both cases for both milk and fat.

Table 9 gives the results by season of calving for first and all lactations.

TABLE 9
Lactation means by season of calving

Season	Fat (2×, 305-day, M.E.)		Milk (2×, 305-day, M.E.)	
	Chlortetracycline	Control	Chlortetracycline	Control
	First lactation			
M-A [a]	464 (6) [c]	371 (10)	12,516 (6)	9,568 (10)
S-F [b]	420 (11)	439 (7)	10,310 (11)	12,109 (7)
Av.	435 (17)	399 (17)	11,088 (17)	10,615 (17)
	All lactations			
M-A [a]	414 (15)	388 (18)	11,149 (15)	10,369 (18)
S-F [b]	403 (15)	431 (14)	9,913 (15)	11,511 (14)
Av.	409 (30)	407 (32)	10,531 (30)	10,869 (32)

[a] March through August.
[b] September through February.
[c] The numbers in parentheses are the numbers of animals in each subclass.

These means indicate a treatment-by-season interaction, in that chlortetracycline was more beneficial in the period March through August than in September through February, while the reverse was true for the controls.

Disproportionate subclass numbers resulted when the chlortetracycline-fed animals and the controls were separated into seasonal groupings. The weighted squares of means analysis (Table 10) should give valid tests of significance of treatment differences with disproportionate subclass frequencies. Treatments and breed-season subclasses were considered. The variation between breeds and seasons and their interaction would be included in the breed-season source of variation. The treatment × breed-season interaction would include the interaction due to treatments by breeds and the interaction of treatments by seasons as well as the three-factor interaction. Previous analysis indicated the second of these to be the only one of importance. The results in Table 10 show that chlortetracycline gave a beneficial effect on first-lactation fat only.

TABLE 10

Weighted squares of means analysis–mean squares (2×, 305-day, M.E.)

	First lactation			All lactations	
	d.f.	Milk	Fat	d.f.	Milk	Fat
Treatment	1	542,527	12,172*	1	322,758	1,539
Breed-Season	6	37,221,397**	6,842*	7	61,133,776**	13,713**
T × B-S	6	3,231,218	5,790	7	3,991,658	2,557
Error	19	2,207,475	2,468	46	2,412,404	3,723

* $P < .05$.
** $P < .01$.

The 40 animals in the experiment left the herd for a variety of reasons. Six animals from the antibiotic group and seven of the controls were sold for dairy purposes to make room for heifers in the milking herd. Five of the antibiotic-fed animals and six of the controls were sold because of low production. Five of the antibiotic group were sold because of failure to conceive and two others were sold after aborting, including one in its first pregnancy. Three of the control group, including one heifer, were sold because they failed to conceive, another because of a malignant tumor, and one heifer was sold as a brucellosis reactor before parturition. One of the chlortetracycline-fed animals and two of the controls were sold because of mastitis. One animal in the antibiotic group died as a result of bloat.

Although data on the reasons for removal of animals from this experiment are presented, culling procedures employed make it difficult to evaluate treatment effect on these removals. It does seem reasonable to assume, however, that there was no appreciable difference between groups in reproductive performance of the animals in this experiment.

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VARIATION IN MILK FAT PRODUCTION IN STATE-OWNED HERDS IN IOWA [1, 2]

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SUMMARY

This investigation was of 12,623 records made by 4,487 cows from 1940 through 1956 in 12 state-owned herds in Iowa.

Herd differences and herd-by-year interactions were responsible for about 6 and 10% of the total variance, respectively. Years, seasons, and the other interactions among these three mainly environmental factors were of minor importance individually; together they accounted for 4% of the total variance.

The records were corrected for the major environmental effects by taking each record as a deviation from the average of all records made in that herd in that same year. Then, for obtaining sire and dam components, etc., only the cows whose sires and dams each had at least two daughters in the herd were used. This analysis involved 7,003 records by 2,398 daughters of 295 sires and was made in three different ways: (1) hierarchal classification of sires, dams within sires, etc.; (2) full sister analysis; and (3) cross classification by sires and by dams. Four times the sire component in the first method gave 0.16 as the intraherd heritability of differences in butterfat production. By the second method, twice the full sister component gave 0.12 as the estimate. By the first method 1.6% of the total variance was attributed to dominance deviations and other things, such as maternal effects, that make full sisters resemble each other more than twice as much as paternal half-sisters. In the cross classification, the component for sire-by-dam interaction was 1.8%. These results suggest that deviations from the additive scheme of inheritance are unimportant, but these estimates of nonadditive genetic effects were not precise.

Butterfat production, as exhibited by an individual cow, is the response of its genotype for this characteristic under the particular environment in which the individual is raised and makes the records. When a population is subjected to selection, its average genotype changes only slowly within short periods of time because: (1) the number of gene pairs involved is large, (2) selection among females can not be intense, and (3) the selections can not be as accurate as they can be with characters less affected by environment and with characters phenotypically expressable in males, also. During recent decades, milk production per cow in the U.S.A. has increased progressively; for instance, from 4,167 lb. in 1924 (18) to 6,438 lb. in 1959 (19). This advance has surely been brought about by improving both the genetic merit of cattle and their feeding and management. One can not specify certainly the relative role of these factors.

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Earlier breeding techniques have been essentially mass selection within a purebreed, with an uncertain amount of attention to pedigrees and performance of relatives, particularly in sire selection. If heritability is high for a certain characteristic, mass selection is quite effective. Heritability is defined as the fraction of the observed variance which is caused by differences in the average effects of the genes involved. For characteristics of low heritability, mass selection is much less effective. It is expedient then to resort more to other breeding techniques, such as using family averages in addition to the individual's own characteristic in predicting the breeding value of that individual. With increased need of complex breeding techniques, more reliable estimates of the different sources of variation in production under different circumstances become increasingly desirable. The purpose of this investigation was to estimate the different sources of variation in lactation records of butterfat production in the state-owned herds in Iowa, a population which may be (but perhaps is not) somewhat different from most D.H.I.A. and H.I.R. herds.

DATA

These data came from 12 state-owned herds in Iowa which, by their location, represented slightly different soil and climatic conditions. The herds were in the Herd Improvement Registry testing program and all cattle included in this investigation were purebreds, registered with the Holstein-Friesian Association of America. The butterfat yields made during the period 1940 to 1956 were adjusted (9) to mature equivalent, twice-a-day, 305-day lactations. From the 12 herds, 12,623 complete records of 4,487 cows were available. The data were tested for normality of the distribution and for homogeneity of the variance among the 12 herds. Some kurtosis resulted from a slight excess of records near the mean and the ends, with a corresponding depletion in the flanks. Some skewness came from a slight asymmetry with an excess of records larger than the mean. Also, the variances differed from herd to herd a bit more than is likely to have been caused entirely by sampling errors. These departures from the simple statistical models seemed too slight, however, to have affected the subsequent analyses noticeably.

METHODS AND RESULTS

One purpose of the investigation was to measure the main environmental effects and the interactions among them. The most obvious possible sources of differences between records standardized for length, age, and times milked per day are herds, years, the season of the year in which the record began, individual hereditary differences between cows, and permanent effects of subclinical disease or injury or other environmental circumstances. Factors, thought to be of minor importance from earlier studies, such as calving interval, dry period, and lactation number (beyond what is included in age) were ignored. Thus, the analysis of extraneous and identifiable factors was restricted to three major sources: herds, years, seasons, and the interactions among them. The records were classified somewhat arbitrarily into seasons in three different ways: (1)

month of calving, (2) meteorological seasons (winter, spring, summer, and fall, i.e., January 1 through March 31, etc.) of calving, and (3) two 6-mo. periods, April to September as one, and October to March as the other.

Of the 12,623 records used, almost one-third were first lactations. Second and later records were made by cows that had escaped any culling that may have been done on first records. If the intensity of selection varied from year to year or from season to season, this could lead to some confounding of the effects of selection with the effects of year and possibly even with the effects of season. To avoid this source of confusion, it was decided also to estimate these effects, using only the first records. Table 1 shows that the results were not very different, although year was a bit more and season a bit less important in the first records.

In the present investigation, Henderson's Method I (5) of obtaining components was used. All variables were considered random. After the effects of the major environmental factors were estimated, the records were corrected for these environmental factors considered important and then estimates of genetic sources of variation were obtained, using both original and corrected records.

The analysis in Table 1 implies that the effects of herds, years, and seasons

TABLE 1

Components of variance expressed as percentages of the total

	All records			First records	
	Seasonal class			Seasonal class	
Source	Month	Season	Half-year	Season	Half-year
Herds (H)	5.3	5.2	5.0	5.9	5.8
Years (A)	0.3	0.3	0.1	1.1	0.9
Seasons (S)	1.8	1.5	1.8	0.2	0.4
H × A	11.1	10.5	11.1	10.0	10.8
H × S	0.7	0.2	0.5	1.0	0.6
A × S	0.3	0.2	0.5	0.3	0.5
H × A × S	0.8	2.4	0.0	1.0	....
Remainder	79.7	79.6	81.0	80.5	80.9

are independently distributed. Many of the cows had two or more records, which keeps adjacent years from being completely independent in the analysis of all records. With the analysis restricted to first-lactation records, this difficulty does not arise. The five sets of components of variance obtained with all records, or with first records only, and classified by different seasonal divisions, are given in Table 1. Only the differences between herds and the interactions between herds and years were of much consequence as sources of variation in butterfat production.

In an attempt to separate most of the effects of steady time trends from the effects of irregular year-to-year changes (the year components of Table 1 contain both), first records were divided into four periods of four to five consecutive years each. The component of variance for the differences among years within these groups measures the irregular year-to-year changes and a little of any steady time trend that may have existed. This component was substantially

zero, whether season was classified into half years or quarter years. Therefore, the values of 1.1 and 0.9 found for year were due almost wholly to a time trend which, of course, could have been caused by genetic improvement or by changes in environment or by both.

The average lactation production per herd varied from 350 to 431 lb., with the over-all production being 410 lb. The analysis with first records presented much the same picture, although the actual averages were slightly higher. Most of the range between herd averages was caused by unusually low production for two herds. The average production per year varied from 389 to 432 lb., with most years averaging between 400 and 420 lb. The situation with first records was only slightly different.

Average production by months in which the lactation began varied from 390 to 427 lb. when all records were used. The lower figures occurred in the spring and summer months. The number freshening was a little lower in the spring months (down to 817 in May), but varied only from 1,042 to 1,172 in the 9 summer, fall, and winter months. Average production by 3-mo. seasons of calving ranged from 400 lb. in the summer to 424 lb. in the fall, when all records were considered. The situation with first records was about the same, except the averages were slightly higher and varied less with season. When all records were used, the warm period, which is the pooling of the spring and summer 3-mo. seasons, averaged 401 lb. in contrast to 418 lb. for the cool period. For first records this range was only from 416 to 424 lb.

If the environmental factors mentioned above were not randomly distributed, their effects could bias the estimates of the genetic parameters. To avoid this, the effects of herd and of herd-by-year interactions were removed from the records by taking every record as a deviation from the average of all records made in that herd in the same year. This method restricted the study to differences between records made in the same herd and starting in the same year. Differences between herd averages and between averages of the same herd in different years were simply set aside and left unanalyzed. Presumably, the differences thus set aside were mainly environmental in origin, although not wholly so.

Components of variance attributable to sires and to dams were estimated. A few sires and most of the dams had just one daughter each. Since estimating differences among sire's and dam's breeding values from records of just one daughter each would be inadequate, all records that were made by a cow whose sire or dam had just one daughter were discarded from this part of the analysis. The remaining records were analyzed genetically in two ways: first, with the original 305-day M. E. 2 × butterfat production and second, with production corrected for general environmental influences by expressing the record as a deviation from the herd-year average. The results with original records show what happened when important environmental influences were ignored in genetic analyses. Most sires were used in only one herd. Each dam and her daughters were in the same herd. The data from all herds were pooled and the analyses were done in three different ways: first, as a hierarchal classification by sires,

dams within sires, among full sisters, and among records of the same cow; second, a similar hierarchal classification using full sisters only; and third, as a cross classification by sires and by dams.

In the genetic interpretation of Tables 2, 3, and 4, it was assumed that: (a) mating was random; (b) epistatic effects were negligible; (c) no environmental correlation existed between members of a related group (e.g., between paternal or maternal sibs); and (d) sex-linkage is negligible. Among these: (a) is substantially correct; (b) is uncertain, but most of any epistatic effects which may have existed will be included in the component V(C) between cows; (c) will have been nearly true for the corrected records, although probably not for the original records; and (d) is likely to be almost true, since only one of the 30 pairs of chromosomes in cattle carries sex-linked genes.

Hierarchal classification of all cows with maternal sisters. Table 2 shows

TABLE 2

Components of variance in the hierarchal classification

Source	d.f.	Original records		Corrected records	
		Value	Per cent	Value	Per cent
V(S)	283	639	8.2	271	4.0
V(D)	1,740	421	5.4	299	4.4
V(C)	363	1,726	22.2	1,757	25.7
V(E)	4,605	5,001	64.2	4,514	66.0
	Total	7,787		6,841	

the variance components among sires, among dams within sires, among full sisters, and among lactations of the same cow. Four times V(S) is an estimate of the additive genetic variance in the population. The variance due to differences between dams within sires—V(D)—is the covariance between full sisters minus the covariance between paternal half-sisters. Four times V(D) is an estimate of the total additive plus the dominance variance in the population. The dam component minus the sire component is an estimate of one-fourth the dominance variance. V(C) is the variance between full sisters. It should contain half the additive variance, three-fourths of the dominance variance, something more than three-fourths of the epistatic variance, and all the variance caused by environmental effects which varied from cow to cow but affected each cow alike in all her records.

The total variance among the original records was 7,787; the corresponding figure with the corrected records was 6,841. The difference is what was removed by taking each record as a deviation from that year's average within that herd. It is most of the variance which appeared in Table 1 in the components for herds and years and herd-by-year interactions. Much of the contrast between original and corrected records in Table 2 appeared in the component for differences between records of the same cow, because two records from the same cow are nearly always made in different years. Therefore, this intracow component in the original records would always contain an intraherd year differ-

ence. However, both sire and dam components—V(S) and V(D)—are also smaller in the corrected records. Percentagewise they decreased even more than V(E) did. This suggests that V(S) and V(D) in the original records included some environmental components that were eliminated by expressing records as a deviation from the herd average that year. Any such environmental inclusions exaggerate the estimates of genetic components made from the original records. However, expressing every record as a deviation from the herd-year average also removed from the corrected records any mean genetic differences which existed between herds and between years within herds. Presumably these were small but, so far as they existed, they cause the genetic estimates from the corrected records to be slightly too small.

The estimate of additive variance from the corrected records—4V(S)—is 16.0%, whereas the estimate of additive plus dominance variance—4V(D)—is 17.6%, suggesting that dominance variance is probably of minor importance in dairy cattle breeding. The estimate of heritability (narrow sense) is thus 16.0%. Repeatability, which is the total genetic variance plus the variance due to environmental effects which are permanent on all the records of a cow, can be estimated as the ratio, $\frac{V(S) + V(D) + V(C)}{V(S) + V(D) + V(C) + V(E)}$, which was 34.1% in this investigation.

Full sister analysis. Table 3 presents the results when only sets of full sisters were included. Twice the full sister group component—2V(FS)—which is 12.6% in the corrected records, is an estimate of the total additive variance plus half of the dominance variance among contemporary cows in the same herd. The estimate is actually less than the 16.0% obtained for additive variance in the earlier analysis, although it should be larger if there is some dominance. Only about a fourth of the information used in the former analysis was available in this full sister analysis. Thus, the discrepancy could easily be due to sampling error. That the two estimates are fairly close and the second is lower suggests again that dominance variance is of minor importance in breeding for fat production.

The component, V(C), obtained in Table 3 contains the variance caused by permanent effects of individual cow environmental differences, such as effects of mastitis, injuries to udder, etc., plus half of the additive variance, three-fourths of the dominance variance, and three-fourths or more of the epistatic variance. V(C) minus V(FS), is an estimate of the permanent environmental

TABLE 3

Components of variance—including only sets of full sisters

Source	d.f.	Original records		Corrected records	
		Value	Per cent	Value	Per cent
V(FS)	306	908	11.5	438	6.3
V(C)	363	1,567	19.9	1,598	23.0
V(E)	1,275	5,406	68.6	4,921	70.7
	Total	7,881		6,957	

variance plus half of the dominance variance and more than half of the epistatic variance. Subtracting from this difference the estimate obtained earlier (assuming negligible epistasis) for the dominance variance yields 15.9% as an estimate of the combined effects of permanent, environmentally caused differences and a large share of the epistatic variance.

Cross classification. This analysis was of the same information used in Table 2 in the hierarchal classification of all cows with maternal sisters. But the component obtained there for dams within sires is divided here into a dam component and a component for interaction between sires and dams. Separating the variances due to main effects (sire and dam) and those due to interactions would be biased if sire and dam were correlated. A small positive correlation of this kind might be in the present original data, because of a slight trend towards genetic improvement over the period studied. It would tend to make the sire and dam estimates slightly too large and the interaction estimate consequently too small. The inclusion of some environmental effects in the sire and dam components would have similar effects and is likely to have been large enough to have some importance in the original records, where time trends and herd differences had not been removed. Neither of these sources of bias are likely to have been large enough to make any material difference in the corrected records.

If maternal effects or other environmental effects are unimportant, and sires and dams are unrelated, the sire and dam components both are expected to contain one-fourth of the additive variance and less than one-sixteenth of the epistatic variance. A real sire-by-dam interaction means that the production of daughters of a particular sire and dam deviates from what would be expected from the progeny averages of that sire and that dam. If the average production of several daughters of a sire, from a random sample of dams, is 40 lb. of fat above the herd average, and if the average production of several daughters of a dam, by a random sample of sires, is 20 lb. of fat above herd average, then the expected average production of daughters of this particular dam by this sire would be 60 lb. of fat above the herd average. Deviations from the expected result could be due to dominance, to epistatic effects, to environment and genotype interactions, and to sampling errors.

The components obtained from this analysis are given in Table 4. The amount of evidence is much the same as in Table 2, the main difference being

TABLE 4

Components of variance—cross classification

Source	Original records		Corrected records	
	Value	Per cent	Value	Per cent
V(S)	643	8.2	273	4.0
V(D)	458	5.8	173	2.5
V(SD)	− 37	....	126	1.8
V(C)	1,725	22.0	1,757	25.7
V(E)	5,001	63.9	4,514	66.0
Total	7,827		6,843	

that the second line in Table 2 is separated into the second and third lines in Table 4. A purely genetic interpretation of the sire, dam, and interaction components seems much more justified for the corrected records than for the original records, since the latter were more vulnerable to year-to-year changes in environment. The sire-by-dam interaction is 1.8%, a small amount. This suggests again that deviations from the additive scheme of gene effects are probably unimportant. However, the breeding values of dams and of some sires are obtained on the performance of only a few daughters, mostly two. Consequently, the amount of information available concerning the interaction component is too small to guarantee that it was estimated very accurately.

Confidence limits. The components obtained in these analyses are useful so far as one can infer from them the parameters in the population which the sample represents. If the distribution of the variances were normal, in addition to the other assumptions made, confidence limits on parameters in the population can be obtained from these estimates as, for example, by the method of Bross (1). The confidence limits of the components shown in Tables 2 and 3 are given in Tables 5 and 6.

TABLE 5

Confidence limits—hierarchal classification

Component	Value	Variance of component	Standard error	95 per Cent confidence limits
V(S)	271	8,146	90.2	94– 448
V(D)	299	65,432	255.8	0– 800
V(C)	1,757	70,344	265.2	1,237–2,277
V(E)	4,514	8,846	94.1	4,330–4,698

Osborne and Paterson (13) give a method of getting the variance of heritability estimates, which are ratios of components of variance. The 95% confidence limits of the heritability estimate thus obtained from the sire component are 6 and 26%. Similarly, the 95% limits on the estimate obtained by using the full sister records, which includes half of the dominance variance, are 1 and 24%. These confidence limits and those in Tables 5 and 6 should be viewed with considerable caution, since it is not certain that the variance components are independent and little is known about their distribution.

DISCUSSION

Differences from herd to herd and from year to year, mostly environmental, were about 16% of the total in Table 1. Such differences are a major source

TABLE 6

Confidence limits—full sister classification

Component	Value	Variance of component	Standard error	95 per Cent confidence limits
V(FS)	438	49,890	223.4	0– 876
V(C)	1,598	74,924	273.7	1,062–2,134
V(E)	4,921	37,927	194.7	4,539–5,303

of error in proving bulls in natural service, because they tend to be alike for all daughters of a bull and do not diminish as the average is based on more and more daughters. When a bull is proved artificially his daughters are usually in many herds and this source of error becomes more nearly random from daughter to daughter. For example, if the sire component is 4% and the herd and year components total 16%, as was approximately true in these herds, but all the rest of the variance was random among paternal sisters, then increasing the number of daughters makes the correlation between sire's breeding value and his daughter average approach $\sqrt{.20}$ as a limit if all his daughters are contemporary in one herd, but it can approach 1.0 if each daughter is in a different herd. Also, in the former case most of the gain in accuracy is achieved with the first few daughters and little more is accomplished by making that number much more than five, while in the latter case considerable accuracy can still be gained by increasing it even past 20. Points such as these are considered in some detail by Lush and McGilliard (11) and by Carter (2).

In theory, these environmental effects which are alike for all daughters of a sire but vary from group to group could be reduced either by preventing the environmental differences from occurring or by correcting the data subsequently. The former is usually not completely practical, either because of physical obstacles or because the cost is prohibitive. In Denmark, testing stations were planned to progeny-test bulls under a standardized environment in which daughters from different herds could perform, yet Robertson and Mason (16) and Touchberry (17) found extra variation between progeny groups at the stations that was not repeated in the field. Correcting the data for the effects of uncontrolled environment is usually cheaper but always incomplete. The group effects are not always identified and, even when the environmental differences are known, the proper corrections for them are always at least a little uncertain.

The 16% found here for herd differences and herd-by-year interactions is smaller than has been found in most studies (4, 6, 14, 15). However, Johansson and Hansson (8) found only 6.4% for the herd differences among 13 herds of Swedish Red and White cattle over a 15-yr. period. This corresponds to the general herd component of between 5 and 6% here. That the differences among herds here were smaller than usual seems to need explaining. The 12 herds here were all fairly large and 11 of them were under a single administrative control for most of the period. This may have standardized the management and feeding more than in the general D.H.I.A. population of Iowa. Another conjecture is that, since their main purpose was to supply milk for use by the inmates of these institutions, rather than to make a profit, the feeding and care of the cows may not have been allowed to vary with feed prices, the seasonal need for diverting labor to other farm enterprises, etc., as much as happens on farms which are geared more closely to prices and costs and which set less importance on steadiness of output.

The corrected first lactations averaged about 15 lb. higher than other records. Possibly, the early records of a cow are over-corrected for age. It is also possible

that many of the records made later in life are affected by adverse conditions, such as subclinical mastitis, or other udder damage, nutritional depletion by the previous lactation, etc. The values of most components when all the records were used agreed closely with those when only the first records were used, suggesting that differences between first and other records did not have serious effects on the analysis.

The mean square remaining after herd, year, and season differences and their interactions were removed was about 7% larger when all records were used than it was when only the first records were used. The difference was too large to be attributed reasonably to sampling errors alone. The explanation may lie in the fact that the first records are not affected by variations in such factors as previous calving interval, length of previous dry period, depletion of nutritional reserves by previous production, etc.

Additivity of effects and independence of variables were assumed in estimating the components. The cows mated to the same bull in any herd are always correlated a little, since they are contemporaries and some are half sisters or otherwise related, slightly, but the environmental part of this would mostly have been removed by expressing the records as deviations from the average of that herd in that year. A little genetic correlation might remain from such practices as mating the daughters of one sire to one bull and the daughters of other sires to another bull. Freeman and Henderson (3) found an average relationship of 0.07 among contemporary animals in 11 commercial herds in New York. In each herd in the present investigation, two or more sires were in use at nearly all times and this relationship would be less. A relationship of 0.07 between dams, but zero between sire and dam, would make the covariance between half-sisters contain $\frac{1.07}{4}$ of the additive variance. Then, the sire component should have been multiplied by 3.7 instead of 4 to estimate the additive variance. Thus, if the sires' mates were related to each other, the estimate of heritability as obtained here from the sire component is slightly higher than it should be. However, the difference would be small and the relationship between the mates of a bull would surely be less than .07 in the present data. The effect of this correlation between dams can probably be ignored with little error.

The 12th herd had been closed to outside breeding for some 20 yr., but was operated as a single population with no attempt to subdivide it into separate inbred lines. The inbreeding was slight, the average having reached only 11.8% among the cows which calved in 1954. It would tend to make the additive variance in the intraherd components only (1-F) as large as if the herd had not been closed. Therefore, in this herd the sire component should have been multiplied by 4.5 (instead of 4) to estimate what the additive genetic variance would be in a noninbred population (20). Other herds in the investigation were of about the same size, but were not closed to outside breeding and inbreeding was avoided in them.

The genetic analysis utilized the records of only those cows whose dam had

at least two daughters. This can not have introduced any serious bias into the analysis, since this criterion eliminated cows whose dams remained in the herd for several lactations but had only one daughter come into production, as well as cows whose dams were culled because of low production in their first lactation.

The numerous heritability estimates obtained for butterfat production in dairy cattle vary somewhat. A few workers have offered evidence that heritability estimates differ depending on the production level of the herds. In many cases, the differences in heritability estimates are so small that they could be due to sampling errors. The numerator, which is the covariance between relatives, may in some studies still include some covariance from environmental differences. If real differences in heritability do exist, several explanations are possible. The environmental part of the total variation in fat production may differ under different circumstances. Then, the denominator of the ratio would vary and this could be responsible for at least small differences in the estimates. In a study by Johansson, using cows with five records each (7), the total mean squares in fat production were 4,066 and 6,277 for low herds and high herds, respectively. Both figures are considerably less than the total variance of over 7,000 found in the present investigation. However, the coefficient of variation was 25.4% in the low herds and 22.5% in the high herds in the Swedish data and 21.9% in our investigation.

This investigation suggests that deviations from the additive scheme of inheritance are unimportant for fat production. Freeman and Henderson (3) calculated, from records of 1,210 Holstein animals in 11 commercial herds, that the average correlation for dominance deviations between related but noninbred cows was 0.007. From this, they concluded that the dominance portion of the total variance would contribute little to the total genetic covariance between related animals in commercial dairy herds, even if the total amount of dominance variance were large.

In these data, as in most investigations, much of the total variance (in this case more than 50% of it) remains unexplained. Part of this could be due to genotype and environment interaction. However, earlier work suggests that such interactions are small. Hickman and Henderson (6) found that 2.4% of total variance was due to herd-by-sire interaction. Wadell and McGilliard (21) found negative values for the same. Legates, Verlinden, and Kendrick (10) concluded from rather extensive data collected throughout the United States that herd-by-sire interactions were not of major importance. Much of the variance left unexplained may be due to micro-environmental influences, as discussed by McBride (12). If these unknown factors are distributed at random, the estimates of the factors considered in this investigation remain unbiased; otherwise, these estimates include parts of the effects of these unknown factors, depending on the kind and extent of the nonrandomness.

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EFFECT OF GLUCOCORTICOIDS ON THYROID FUNCTION OF CATTLE[1]

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SUMMARY

The influence of two glucocorticoids upon the thyroidal-I^{131} release rate of cattle is reported. After the normal release rate of each animal was determined during a five-day period (recycling of I^{131} blocked by a goitrogen), seven cows were injected with hydrocortisone at the rate of 75 mg/100 lb. body weight per day for six days. Of these cows, five showed varying degrees of depression of thyroidal-I^{131} release, whereas two showed an increased release rate. Meticorten, a synthetic glucocorticoid, injected in eight heifers at the rate of 40 mg/100 lb. body weight per day, showed a variable increased release rate in six animals, whereas two showed a slight depression. These data suggest that hydrocortisone at the level administered tends to depress thyrotropin and thyroxine secretion, whereas meticorten tends to stimulate thyroid function. The causes of the individual differences in response in cattle to these glucocorticoids is not known, but may be related to individual secretion rates of other hormones. It is suggested that cattle with low thyroxine secretion rate, which is further depressed by increased secretion of hydrocortisone associated with parturition, would show low PBI and high 17-hydroxy-corticosteroid values characteristic of cows with ketosis.

The relation of the pituitary adreno-corticotropic hormone (ACTH) to the regulation of secretion of the glucocorticoids has been considered generally to be related to the phenomenon of stress and to that extent unfavorably influences both growth and milk secretion. However, it should be recognized that the hormones of the adrenal cortex are essential for life, for upon adrenalectomy survival is of short duration without replacement therapy. Thus, the secretion of the hormones of the adrenal cortex are of fundamental importance in normal animals.

Further, the adrenal cortical hormones play an important role in the lactation process. In hypophysectomized animals, lactation can be initiated (7, 13) or maintained (5) only by the simultaneous administration of lactogen and ACTH or the adrenal cortical hormones.

The adrenal glucocorticoids received their name from their capacity to cause the deamination of amino acids. Following the removal of the NH_2 group, the carbohydrate residue of many amino acids can be converted to glucose in the liver. As a result, the blood glucose can be increased and, thus, glucocorticoids are useful in the treatment of ketosis in cattle (23), or the excess glucose can be stored in the body as glycogen. The deamination of amino acids, however, decreases their availability for somatic growth or the synthesis of milk proteins and thus depresses growth and milk secretion.

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In our study of factors influencing the thyroxine secretion rate of dairy cattle, it has been shown that estrogen at a level of .3 mg/day/100 lb. body weight stimulated a significant increase in thyroidal-I^{131} release rate (15). In a further study, estrogen was shown to increase thyroxine secretion rate (16). These data suggest that the increased secretion of estrogen in late pregnancy (6) not only stimulates increased secretion of lactogenic hormone, but thyrotropic hormone and thyroxine as well.

It has been suggested by some that parturition is a stressful condition and as such it would be expected that increased release of ACTH and increased secretion of glucocorticoids would be stimulated (21). Brush (4) reported a rise in plasma glucocorticoids in some cows a few days before calving, in one case in association with a difficult parturition, and in others for several days after calving. In spite of the possible increase in glucocorticoids postpartum, ketosis occurs most frequently during the first 3 wk. following calving.

Robertson *et al.* (19, 20) noted that plasma 17-hydroxycorticosteroid values of cows with ketosis were significantly higher than for normal cows, whereas the PBI values were lower for cows with ketosis than for normal cows. These observations are of significance in the light of the observation by Reece (18) that thyroprotein was of value in the treatment of ketosis. The beneficial effect of thyroxine in ketosis may be due to the increase in blood sugar observed by Graham *et al.* (8) and Smith and Dastur (24) upon the administration of thyroxine.

These data are of interest and significance in relation to the general problem of the interrelation of the adrenal cortex and its hormones and the thyroid gland and thyroxine secretion. Money (11) and Ingbar and Freinkel (10) have reviewed the literature in this field.

During recent years, with I^{131} available, three indices of thyroid gland function have been suggested; namely, I^{131} uptake, thyroidal-I^{131} release rate (with and without recycling depressed with a goitrogen), and thyroxine secretion rate determination.

The literature concerning the influence of either ACTH or the glucocorticoids in respect to thyroid function is very confusing. The majority of the studies indicate that these compounds depress or have no influence on I^{131} uptake. In regard to thyroidal-I^{131} release rate, studies indicating a decreased release rate have been reported by Myant (12) in the rabbit, Brown-Grant (3) in the rat, Sherer and Siefring (22) in man, no effect by Perry (14) and Albert *et al.* (1) in the rat, and an increase by Beck (2).

Due to the lack of uniformity in the literature in regard to the role of glucocorticoids in thyroid function, it seemed desirable to study their influence in dairy cattle. It was hoped that the observations would contribute to an understanding of the influence of the stress of parturition upon thyroid function at this time.

EXPERIMENTAL METHODS

Nonlactating, mature, and immature dairy animals of several breeds were given 300 μc. of carrier-free NaI^{131} intravenously. After maximum uptake of

I^{131} by the thyroid gland (three days) 24 g/day thiouracil was given by gelatin capsule to prevent recycling, and daily measurements of thyroidal-I^{131} were made (17) for a five-day control period. Hydrocortisone (cortisol-hydrocortone) at 75 mg/100 lb. body weight or meticorten at 40 mg/100 lb. was administered subcutaneously during a six-day test period. Potassium chloride was administered at the rate of 14 g. per 1,000 lb. body weight to prevent excess potassium loss due to the glucocorticoids.

Release rates of thyroidal-I^{131} were calculated according to the method previously described (15), with the term k″4 being employed for release rate constant per hour during thiouracil administration. For comparison, the daily percentage release rate is presented. These values indicate the percentage of the total I^{131} present in the thyroid glands which is released each 24 hr. Comparison of the release during the control period, with the release during the administration of the glucocorticoids, indicates the effect of the hormone on the TSH secretion and, indirectly, on the secretion of thyroxine.

RESULTS

The two chief glucocorticoids secreted by the bovine adrenal gland are hydrocortisone and corticosterone in a ratio of 1:1(9). Hydrocortisone should, therefore, be representative of the natural hormone secreted by cattle when stimulated by ACTH. The release rate of thyroidal-I^{131} from the gland, when the recycling of I^{131} is prevented by a goitrogen, follows an exponential decline (a straight line when plotted on semilog paper). The release rate is believed to be dependent upon the rate of discharge of thyrotropic hormone (TSH). If hydrocortisone influences the rate of secretion or discharge of TSH, then the release of thyroidal-I^{131} will be increased or decreased accordingly.

It will be noted that hydrocortisone, at the level administered, tended to depress thyroidal-I^{131} release to varying degrees in five of the seven cows (Table 1). In two other cows, there was a slight increase in one and a more marked increase in the second. These data indicate considerable variability in individual response in dairy cattle to hydrocortisone, although in the majority of the animals the tendency was to inhibit thyroidal-I^{131} release, which indicates depression of TSH secretion or release and, in turn, depression of thyroxine secretion.

Meticorten (Prednisone). This is a synthetic cortisone-like compound produced by dehydrogenation at Positions One and Two of the cortisone nucleus (Figure 1). It has gluconeogenic action comparable to hydrocortisone but is claimed to be more potent. It has been suggested for use in treatment of ketosis in dairy cattle.

In contrast to the effect of hydrocortisone, meticorten stimulated a variable increased release of thyroidal-I^{131} in six of eight experimental animals. Two animals showed a slightly depressed release rate (Table 2). These observations indicate that meticorten tends to increase thyroxine secretion in most, but not all, animals.

Paired "t" tests of data (Tables 1 and 2) indicate with both compounds a lack of change of statistical significance. However, it is believed that the obser-

TABLE 1

Effect of hydrocortisone on thyroidal-I^{131} release rate

Animal No.	Breed	Body weight (lb.)	Month TSR determined	TSR** (mg/day/100 lb.)	Thyroidal-I^{131} release rate—recycling blocked with thiouracil		
					Hydrocortisone (administered in April)		
					Control period k″4	Period k″4	Difference***
38	Guernsey	1,300	December	0.4	0.00614	0.00419	−0.00195
					(14.7%)*	(10.0%)	(4.7%)
601	Holstein	760	February	0.5	0.00417	0.00261	−0.00156
					(10.0%)	(6.3%)	(3.7%)
3625	Guernsey	1,125	February	0.4	0.00760	0.00610	−0.00150
					(18.2%)	(14.6%)	(3.6%)
348	Jersey	937	February	0.3	0.00853	0.00732	−0.00121
					(20.4%)	(17.5%)	(2.9%)
160	Jersey	1,197	February	0.3	0.00624	0.00580	−0.00044
					(14.9%)	(13.9%)	(1.0%)
3596	Guernsey	1,200	February	0.6	0.00210	0.00310	+0.00100
					(5.04%)	(7.4%)	(2.4%)
H_2	Holstein	456	February	0.5	0.00450	0.00770	+0.00320
					(10.8%)	(18.5%)	(7.7%)

* Each value in parentheses indicates the percentage of I^{131} present in the thyroid gland which is released each 24 hr. = k″4 × 24 × 100.

** TSR = thyroxine secretion rate.

*** Paired "t" test indicates a lack of change of statistical significance. Physiological effect of hydrocortisone is believed to be significant in each animal.

TABLE 2

Effect of meticorten on thyroidal-I^{131} release rate

Animal No.	Breed	Body weight (lb.)	Month TSR determined	TSR** (mg/day/100 lb.)	Thyroidal-I^{131} release rate—recycling blocked with thiouracil		
					Meticorten (administered in May)		
					Control period k″4	Period k″4	Difference***
264		650	February	0.5	0.00655	0.01174	+0.00519
					(15.7%)*	(28.2%)	(12.5%)
601	Holstein	760	February	0.5	0.00699	0.01147	+0.00448
					(16.8%)	(27.5%)	(10.7%)
H_1	Guernsey	400	February	0.4	0.00660	0.00950	+0.00290
					(15.8%)	(22.8%)	(7.0%)
38	Guernsey	1,300	December	0.4	0.00240	0.00380	+0.00140
					(5.8%)	(9.1%)	(3.3%)
41	Guernsey	1,250	November	0.5	0.00180	0.00310	+0.00130
					(4.3%)	(7.4%)	(3.1%)
260	Holstein	650	February	0.5	0.00263	0.00288	+0.00025
					(6.3%)	(6.9%)	(0.6%)
263	Holstein	780	February	0.5	0.00400	0.00358	−0.00042
					(9.6%)	(8.6%)	(1.0%)
265	Holstein	850	February	0.4	0.00435	0.00331	−0.00104
					(10.4%)	(7.9%)	(2.5%)

* Each value in parentheses indicates the percentage of I^{131} present in the thyroid gland which is released each 24 hr. = k″4 × 24 × 100.

** TSR = thyroxine secretion rate.

*** Paired ''t'' test indicates a lack of change of statistical significance. Physiological effect of meticorten is believed to be of significance in each animal.

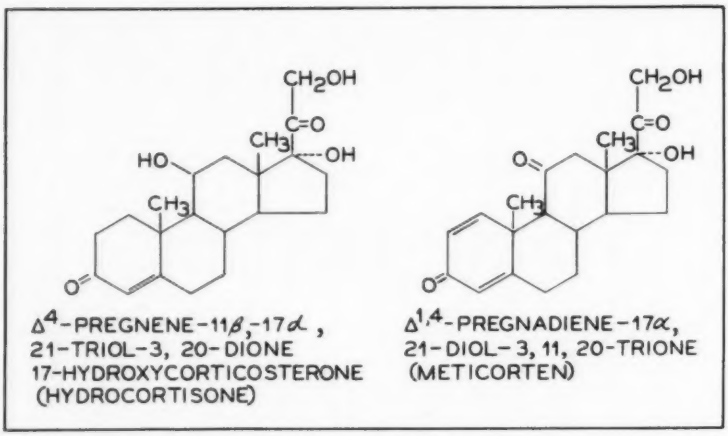

FIG. 1. Structural formula of hydrocortisone and synthetic glucocorticoid, meticorten compared.

vations are physiologically significant in spite of the variation of response observed. It s suggested that individual animal differences in the interaction of the various hormones play a role in the observed results. Thus, it is possible that the glucocorticoids synergize with other hormones in producing effects upon TSH secretion. If such hormones are secreted in optimal amounts the glucocorticoid response might be increased, whereas if other hormones are secreted at low levels the response might be minimal or reversed. This possibility will be explored.

DISCUSSION

The relation of the adrenal glucocorticoids to the functional activity of the thyroid gland is of great interest. Is there a tendency for these hormones to stimulate or depress thyroxine secretion? Under situations of stress, where it is expected that increased secretion of the glucocorticoids would be observed, the stressful condition itself might influence thyroxine secretion as well. In the present experiment, the direct administration of the glucocorticoid should indicate its uncomplicated effect on thyroid function.

These data show that hydrocortisone tended to depress thyroidal-I^{131} release and TSH and thyroxine secretion in most of the animals. The cause of the variability in response of the individual animals is not evident. The normal thyroxine secretion rate of each of these animals is presented (Table 1). It will be noted that the two animals which showed increased thyroidal-I^{131} release were relatively higher secretors of thyroxine, but one animal in the other group secreted thyroxine at the same level.

Our data suggest that at the time of parturition thyroxine secretion tends to be increased as a result of estrogen. To the extent that increased secretion of glucocorticoids is stimulated by stress or other related conditions, an opposing depression of thyroxine secretion might occur in many animals. Thus, in cows

with a low normal thyroxine secretion, the further depression of thyroxine secretion by hydrocortisone associated with parturition might be a factor in the etiology of ketosis at this time.

In other words, physiological ketosis might be due, primarily, to a low normal thyroxine secretion rate, further depressed by increased secretion of the adrenal glucocorticoids. If this is true, then ketosis would be favorably influenced by thyroxine or by the feeding of thyroprotein. This would explain the favorable report of Reece (18). Since ketotic cows show a low blood sugar level and Graham *et al.* (8) and Smith and Dastur (24) showed that thyroxine increased the blood sugar level of cows, the low thyroxine secretion rate could have a depressing effect on blood sugar. If this is true, the restoration of normal blood thyroxine levels would have a favorable effect on blood sugar. This would be augmented by the effect of the glucocorticoids in their capacity to provide blood glucose from deaminated amino acids.

The observation that meticorten tends to stimulate an increased release of thyroidal-I^{131} (and thyroxine secretion) in six of eight cows is quite surprising in relation to our observations on hydrocortisone. Both compounds have gluconeogenic properties and react similarly in respect to other biological functions. It is possible, of course, since both compounds show variability in response from depression to stimulation of thyroidal-I^{131} release, that no true difference exists between the compounds and that the variation observed is due to as yet unrecognized differences in the endocrine balance of the animals, causing in some a stimulation and in others a depression of thyroid function.

The present data, however, indicate that meticorten tends to stimulate thyroxine secretion and, if so, would have a more favorable action in the treatment of ketosis, to the extent that low thyroxine secretion is related to the etiology of ketosis.

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TECHNICAL NOTES

OBSERVATIONS ON AMMONIA AND VOLATILE AMINES IN MILK[1]

Ammonia is recognized as a trace component of fresh milk (1, 4, 5). However, no information is available concerning the possible presence of trace amounts of free volatile amines in fresh milk. This investigation was made with the following objectives in mind: (a) to develop procedures for quantitative extraction and chromatographic analysis of ammonia and volatile amines from milk, and (b) to apply these methods for the determination of ammonia and volatile amines in milk of varying flavor quality. For the purpose of the latter objective, fresh milk samples which possessed either good or feed flavors were subjected to solvent extraction and the free ammonia and volatile amines thus obtained were analyzed by gas-liquid chromatography.

The extraction procedure, developed experimentally, was as follows: (a) a 20-ml. aliquot of acid whey was made alkaline to phenol red with *N* NaOH; (b) 100 ml. of ethyl alcohol, 200 ml. of ethyl ether, and 200 ml. of petroleum ether were added to the whey in a separatory funnel and the contents shaken vigorously and separated; (c) the extraction procedure was repeated twice, except that only 50 ml. ethanol was used in the second extraction and none was used in the third extraction; (d) the extracts were combined and acidified with 0.1 ml. of concentrated hydrochloric acid to form nonvolatile chloride salts; (e) the solvents were evaporated under a hood and the residues were dried in a calcium chloride desiccator for 8-12 hr.; (f) the dried samples then were dissolved in a minimum amount of ethyl ether, transferred to a 10-ml. volumetric flask, and two drops of distilled water were added; (g) after vigorous shaking, 0.3 g. crushed sodium hydroxide pellets were added and the flask was shaken vigorously again; (h) the volume within the flask was adjusted to 10 ml. with ethyl ether and a 5-ml. sample was used for chromatographic analysis.

The gas-liquid chromatographic technique used was a modification of that of James *et al.* (3) and Hankinson *et al.* (2). A 6-mm., 48-in. glass column was packed with acid-treated Celite 545 and a fixed liquid phase (20%) consisting of 85% undecanol (Carbide and Carbon Co., Inc., New York) and 15% liquid paraffin. This combination was added to 32 in. of the column and the remaining 16 in. was packed with dry celite. The packed column was pretreated by heating to 130° C. for 30 min.

The sample was added to the column as follows: (a) 0.5 ml. of ethyl ether was introduced and followed by 5 ml. of the sample ether mixture; (b) dry nitrogen was applied at a constant pressure of 60 cm. of mercury. Five minutes after the sample was added, glycerol was circulated through a jacket surrounding the chromatographic column and the temperature of this medium was gradually raised from 25 to 130° C. over a period of 50 min. The compounds eluted from the column were collected in distilled water which had been neutralized with N/100 sodium hydroxide to the end point of phenol red. As the compounds were collected in the distilled water, continuous titrations were made with N/100 hydrochloric acid and burette readings were taken at 1-min. intervals to indicate the amount of volatile akaline compounds eluted.

Analyses of ammonia and individual amines were made to establish retention times and then the method was applied to known mixtures. The temperature gradient, separation, and retention times of a known mixture of amines and average recovery of these compounds from milk is presented in Figure 1

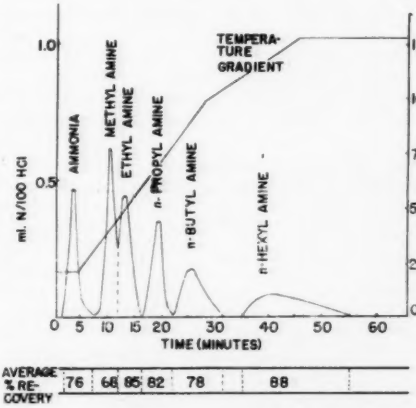

FIG. 1. Temperature gradient, separation of known amines and recovery of these compounds from milk.

for ammonia, methyl, ethyl, n-propyl, n-butyl, and n-hexyl amines. Resolution of this mixture was achieved except for the incomplete separation of methyl and ethyl amines. The recoveries, which were reproducible within ± 5%, ranged from 68% for methyl amine to 88.3% for n-hexyl amine. Separate trials revealed that di-methyl and tri-methyl amines

[1] Technical Paper 14-60, The Department of Dairy Technology, The Ohio Agricultural Experiment Station, Journal Article 51-60. Supported by Hatch Funds through the O.A.E.S.

had the same retention time as methyl amine and that isopropyl amine had the same retention time as ethyl amine.

The method was applied to milk and three compounds were isolated and tentatively identified by retention times as ammonia, n-propyl amine, and n-hexyl amine. Aliquots of these eluates were collected in liquid nitrogen and compared with known compounds by Infra Red spectroscopy using a Beckman IR-5 instrument, which confirmed the tentative identifications.

Subsequently, the ammonia and volatile amine contents were determined for 21 samples of fresh raw milk obtained from individual cows. Eleven of the samples were selected by a panel of four judges as having good flavor and exhibiting no detectable off-flavors. The ten other samples were obtained from individual cows fed chopped meadow crop or silage 1 hr. before milking, and each exhibited a strong feed flavor. The results of analyses of these milk samples for ammonia and volatile amines are in Table 1. Ammonia, n-propyl amine, and n-hexyl amine were found in all samples. The ammonia content ranged from 0.3 to 3.0 μM/10 ml. in good-flavored milk and from 0.2 to 1.5 in the feed-flavored milk. The ranges for propyl amine were from 0.5 to 1.7 μM/10 ml. in good-flavored milk and from 0.9 to 2.5 μM/10 ml. in feed-flavored milk. Values for hexyl amine ranged from 0.5 to 1.7 μM/10 ml. in good-flavored milk and from 0.7 to 2.4 μM/10 ml. in feed-flavored milk. The limited data suggest a possible relationship between the propyl and hexyl amines and feed flavor. Those compounds found in milk and methyl, ethyl and n-butyl amines were added individually to good-flavored milk to determine their effect on flavor. An experienced taste panel of ten members evaluated the samples. The results are presented in Table 2. Several of the amines, especially n-hexyl amine, produced an off-flavor described as green grass, weed, or feed, when present in the concentrations found in feed-flavored milk.

TABLE 1

Ammonia and volatile amines in raw milk

Milk source	Flavor	No. of samples	Ammonia Range	Ammonia Av.	C_1-C_5 Amines Range	C_1-C_5 Amines Av.	C_6 and higher boiling point amines Range	C_6 and higher boiling point amines Av.
Panel selected	Good flavor	11	.3 –3.0	1.1	.5–1.7	0.9	0.5–1.7	1.0
Corn silage fed	Strong feed	4	0.24–0.4	0.4	0.9–2.5	1.7	0.7–2.4	1.4
Grass silage fed[a]	Strong feed	3	0.2 –1.5	1.2	1.4–2.5	1.8	0.9–2.0	1.4
Cut grass fed[a]	Strong feed	3	0.2 –0.5	0.4	1.7–2.4	2.2	1.6–2.2	2.0

[a] Same meadow crop source fed 1 hr. before milking.

TABLE 2

Effect of added amines on milk flavor

Compound added	Concentration level μM/10 ml.[a]	Flavor response
Ammonia	2–3	No effect
	4	Slight medicinal
Methyl amine	1	Metallic
Ethyl amine	1	Medicinal
	2	Slight feed, medicinal
n-Propyl amine	1.7	No effect
	2.2	Slight feed
	2.7	Slight feed
	6.7	Unclean, slight cowy
n-Butyl amine	1.0	No effect
	1.5	Cowy
	3.0	Feed, malty
n-Hexyl amine	1.6	No effect
	2.1	Weed
	2.6	Weed, feed, green grass
	6.6	Chemical medicinal

[a] Corrections made for ammonia, n-propyl and n-hexyl amine content of the milk.

SUMMARY

A gas-liquid chromatography method was developed for milk and applied to good and feed-flavored milks. All fresh milk contained ammonia, propyl and hexyl amines. Feed-flavored milk contained higher concentrations of n-propyl and n-hexyl amines than were found in good-flavored milk. Addition of these amines to milk revealed that they caused a weed or feed type flavor.

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OBSERVATIONS ON THE ACCURACY OF THE GRADUATIONS OF BABCOCK SKIMMILK TEST BOTTLES

Some time ago, we were asked if the instrument we had used to check the accuracy of Babcock milk test bottles could be used to test skimmilk bottles.[1] The bottles were to be used for the measurement of lipids in blood. Such measurements would require some modification of the instrument, but we agreed to try, and we have succeeded in measuring the errors in a set of 24 bottles.

TABLE 1
Errors found in the graduation of 24 Babcock skimmilk test bottles

Per cent marked	Measured value			Standard deviation from marked value
	Mean	Maximum	Minimum	
0.10	.1004	.104	.098	.0015
0.20	.2005	.205	.196	.0025
0.30	.3011	.310	.293	.0035
0.40	.4000	.410	.388	.0044
0.50	.4994	.515	.485	.0055

[1] J. Dairy Sci., 43: 690. 1960.

So far as we know, there are no legal specifications governing the accuracy of such bottles, and no state agency examines skimmilk test bottles for accuracy. For that reason, we thought others might be interested in our data. We believe that the errors in the individual measurements do not exceed 0.2 division and, in most cases, the error is less.

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PRODUCTION OF ISOVALERIC ACID FROM LEUCINE BY *BACTEROIDES RUMINICOLA*

Certain branch-chained volatile fatty acids such as isovaleric acid are essential for the growth of several species of cellulolytic ruminal bacteria (1, 3). Such acids are produced from mixtures of amino acids incubated with the mixed ruminal population (5). It has been suggested that the precursor of isovaleric acid is leucine (4, 5). The species of ruminal microorganisms which convert amino acids to branch-chained volatile fatty acids have not been identified.

Recent work indicates that *Bacteroides ruminicola* plays an important role in production of ammonia from amino acids in the rumen (2). Further studies indicated that ammonia production from single or pairs of amino acids by washed suspensions of *B. ruminicola* subsp. *brevis* (Strain 118B) was erratic and the only amino acids that on occasion gave definitely detectable ammonia production were DL-serine, L-aspartic acid, and L-cysteine. However, casein hydrolysates gave consistently good ammonia production. It was believed that perhaps with more sensitive methods the breakdown of other amino acids could be detected. The present studies were done to determine if this bacterium produces from amino acids any of the branch-chained volatile fatty acids required by the cellulolytic bacteria.

Studies, to determine if the branch-chained acids essential for growth of the cellulolytic organisms were produced, involved the incubation of acid-hydrolyzed casein (6 mg. per milliliter) with a washed suspension of the bacterium for 20 hr. at 37° C. in CO_2-bicarbonate buffer under anaerobic conditions. The acid volatile fraction was separated from the reaction mixture by steam distillation and concentrated under alkaline conditions. The quantity of essential branch-chained volatile fatty acids produced was estimated by microbiological assay, utilizing a medium similar to that of Allison *et al.* (1), but with volatile fatty acids omitted. The assay organism was *Ruminococcus albus*, Strain 7 (1). Similar steam distillations of the reaction mixture in which either casein hydrolysate or cells were omitted served as controls. The results indicated that approximately 1.8 μM per millili-

ter of isovaleric acid-replacing factors were produced by *B. ruminicola* from casein hydrolysate.

Experiments were next undertaken to determine whether leucine was converted to a five-carbon volatile acid. Washed suspensions of the organism were incubated with 1 mg. per milliliter of acid-hydrolyzed casein and DL-2-C^{14} leucine. Aliquots, removed immediately, after 5 hr., and after 24 hr. of incubation, were steam-distilled under acid conditions. The C^{14} present in the residue and distillate was determined with a windowless gas flow counter. All samples were plated at less than 0.5 mg. per $cm.^2$, so that self-absorption was negligible. Measurements are reported as counts per minute (CPM) per 8-ml. reaction mixture.

No radioactivity was present in the acid volatile fraction of the 0-hr. aliquot. After 5 hr. of incubation, the acid volatile fraction contained 48,000 CPM whereas the residue contained 220,400 CPM. After 24 hr. of incubation the acid volatile fraction contained 87,300 CPM and the residue contained 165,400 CPM. Thus, about 18% of the radioactivity from DL-2 C^{14} leucine was found in the distillate after 5 hr. of incubation, and approximately 34% was present after 24 hr. of incubation.

In another experiment, with a more dilute cell suspension, 11% of the C^{14} appeared in the volatile acid after 20 hr. By column chromatography approximately 94% of the total radioactivity present in the acid volatile fraction was detected in the fraction containing valeric acids (6).[1]

These results indicate that a strain of *B. ruminicola* subsp. *brevis* produces a branch-chained volatile acid(s) which is required for the growth of other rumen bacteria, and that at least part of this acid is a valeric acid, presumably isovaleric acid, produced from leucine. This production by a rumen bacterium of a factor necessary for the growth of another rumen bacterium exemplifies the interdependence of the mixed ruminal microbial population.

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[1] The authors are indebted to H. G. Wiseman, who modified the column for more effective separation of 5 and 6 C. acids, and to Milton Allison, whose criticisms have materially aided in this work.

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A SIMPLIFIED METHOD OF OBTAINING DIFFERENTIAL AND TOTAL VIABLE ANAEROBIC COUNTS OF RUMEN BACTERIA EMPLOYING SEALED SMALL-DIAMETER GLASS TUBING[1]

Two major obstacles encountered when attempting to culture rumen bacteria are the difficulty in maintaining anaerobic conditions throughout, and the limited number of samples which can be handled within a reasonable length of time. Sections of small glass tubing (4 mm. outside diameter, cut into 12-in. lengths) are employed in much the same way as are Burri tubes, in an effort to overcome these obstacles. These hold 1 ml. of culture medium and allow about 2 in. of gas space at both ends of the column of medium.

The glass tubing sections are placed in a canister and sterilized in an autoclave before use. A 2-ml. sterile hypodermic syringe, fitted with a sterilized rubber adapter, is used to pull the medium into the tubing. The syringe is first filled with CO_2 gas and then fitted to one end of a section of glass tubing. The other end of the tubing is inserted in the mouth of a test tube containing medium with the desired dilution of whole rumen content, and the CO_2 in the syringe is exhausted through the tubing to remove all air. The end of the

tubing is lowered into the medium and 1 ml. is drawn into the tubing. The end of the tubing is then removed to just above the medium and the medium is drawn about 2 in. into the tubing. The tubing is removed from the test tube and the exposed end immediately sealed by flame. The syringe is disconnected and the other end of the tubing is sealed by flame. Since the filled sections of glass tubing are sealed, incubation is carried out in a water bath held at 39° C. for 72 hr.

Counts of colonies are made, using a wide-field microscope at 30 × magnification. Colonies are counted and grouped according to colony characteristics. They are picked for further study by breaking the glass tubing at the site of the colony. This procedure is used routinely in this laboratory, and counts of total viable anaerobic bacteria ranging through 10^9 per milliliter of rumen content have been recorded.

The Bryant and Burkey (1) RGCA medium and anaerobic dilution solution were employed in this method. Rumen samples were diluted through 10^{-6} or 10^{-7} in the anaerobic dilution solution. The dilution used in filling the glass tubing was made by adding 1 ml. of anaerobic dilution solution to 9 ml. of RGCA medium which had been liquefied and cooled to 48° C. Dilutions of rumen content which gave from 25 to 100 colonies per glass tubing were employed. All unsealed vessels containing dilution solution or liquid medium were kept under a stream of CO_2, which was passed through a washing column to remove traces of O_2 (2).

The advantages of this method are: (a) rapidity of preparation, (b) ease of counting and distinguishing colonies, (c) comparative ease in maintaining anaerobiosis, (d) ease in isolation of colonies for subculture and microscopic observation, and (e) reduction of storage and incubator space.

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

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OUR INDUSTRY TODAY[1]

WORKING WITH INDUSTRY ORGANIZATIONS

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This portion of our program is the result of a direct request from one of our members who wrote the program planning committee saying, "We would also suggest that a panel of experts be assembled to discuss some of the special problems which dairy manufacturing specialists encounter in their work, especially when it becomes necessary to deal with specific pieces of commercial equipment and specific processes developed by commercial people. Also, it would be of great interest to our group to have a discussion of special methods which have proved themselves in practice for dealing with organized groups in the dairy and food industry."

R. B. Redfern

I know that all of you realize there is no common answer to the first portion of the request regarding problems with special pieces of equipment and special processes developed by commercial concerns. But I will venture to say that all of you have this problem. If I may, I would like to change the word problem to opportunity. Because it is just that when a specific piece of equipment or process reduces costs, changes a product in such a way as to increase consumption, or when new procedures and processes encourage use of dairy products. Isn't all of this beneficial? Then why shouldn't individuals dedicated to the development of new ideas, assist this program? If for no other reason we should cooperate in order to broaden our knowledge. Still a greater selfish motive would be to strengthen the ties between our educational facilities and our industries. If we are realistic we will recognize that education and business in its modern sense must be tied together if we are to progress as fast and as economically as we can. In North Carolina we think there is still a better reason. This is to assist industry in such a way that whenever they have a problem they will immediately think of North Carolina State College. In so doing, we can help chart the course of progress in industry and above all maintain good relations with the people who set us up in business. In other words, if it were not for the dairy processing industry, there would be no real need for Dairy Manufacturing Extension Specialists.

Since we have no stock answer to this question, let us cite an example of one specific piece of processing equipment and the methods that were used in North Carolina and which could apply to any state. I have reference to the use of flavor-removal equipment to process fluid milk. At this point, I would like to say that in carrying out a program of this nature there must be close cooperation between research and extension. In this case, it was more of a research project or application of practical research, and the credit should go to the Dairy Manufacturing Section of North Carolina State College. When we analyze the program as it developed, we find that it falls into the following pattern:

1. Defining the problem.
2. Solving the problem.
3. Working with commercial equipment companies.
4. Working with regulatory and public health officials.
5. Convincing our dairy plants that they had a problem, and that we had a solution.
6. Assisting in the installation of and encouraging the use of flavor removal equipment.
7. Continuing to advise and assist when problems develop.

For years in North Carolina, to promote production of milk, we boasted of a 9- to 10-mo. grazing season and at the same time we told farmers to remove their cows from pastures after 1 to 2 hr. of grazing to prevent obnoxious flavors in the milk. We realized that grazing was the cheapest source of feed to the producers; yet, we also knew our customers would not drink the milk if the cows were allowed to graze without restriction. Thus, the problem was to utilize the grass we had been boasting of and still sell the milk. The consumers were not concerned with our grass problem; they were concerned with the flavor.

A solution to the problem would be to let the cows eat the grass and then process the milk in such a way as to remove the flavors. We had known for many years that cream had

[1] The first three papers in this section were presented to the Dairy Manufacturing Extension Section at the 55th Annual Meeting of the American Dairy Science Association, June, 1960.

been vacuum- and/or steam-treated. Could whole milk be treated in the same manner? We knew also that air bubbled through milk after it reached 145° and during a holding time of 30 min. could remove certain volatile flavors, if the vat cover was allowed to remain open. Was such a machine available that could be adapted to HTST pasteurization, or was such a machine available that would pasteurize milk and remove off-flavors at the same time? The vacreator or some similar type machine was a possibility.

Thus, the next step was to work with a commercial company to get such equipment. Since the Vacreator was already being used by the butter industry, it was selected. There were others available, but the Vacreator appeared to have most of the features desired, especially that of steam injection. The use of steam was considered important, because we knew that certain kinds of onion flavors would not volatilize under a temperature of 190° F. This, as you can see, involved a selection of equipment, but no one could criticize us for the problem. Our problem in the main was onion and strong pasture flavors. At all times we kept the local regulatory officials aware of our intentions and problems and got temporary permission to proceed in every instance. We could not have asked for more cooperation. The only thing we had to do was to keep them informed.

We made this selection of equipment with the knowledge that there might be some adverse comments. But we also knew that to obtain data in the shortest length of time it would have to be done this way. The real success of this decision was in the sharing of information with the various equipment companies without divulging trade secrets.

The preliminary work was carried on some 5 to 7 yr. in our college dairy plant and laboratory. The next step was to convince our dairy plants that the problem was theirs as well as the dairy farmers', and to get at least one company to install a machine. In late 1954 one of our larger independent dairies installed a Vacreator. During 1955 this operation was closely observed by producers, processors, and supply men, with each having a different objective. The producer knew that its success could save him money, the processor knew that initially it would cost him money, and the supply men saw sales for thousands of flavor-removal machines. While all of this was going on, we made a survey to learn how much milk was being rejected annually because of off-flavors. A conservative figure showed over $300,000 worth of milk rejected annually which was a direct loss to producers. Processors estimated a 5 to 10% decrease in fluid milk sales in the months of March, April, and May, caused mainly by consumer resistance to the spring flavor. The combined figures showed an estimated loss to the industry of $500,000 annually. These figures we publicized at producer meetings, county agent meetings, dairy processing meetings, etc. The result was that several plants' producers offered to buy the machines for their plant. This, of course, served as the stimulus for several plants to install machines. In 1955, some five dairy plants purchased flavor-removal machines.

At this point, another aspect of cooperation developed among plants. Those plants that had purchased machines allowed other processors to use them to determine whether they would remove the specific flavors contained in their producers' milk. Our facilities at the college also were utilized. At least five companies brought cans of their worst milk with onion to the college dairy plant to be processed. One cooperative manager brought his board of directors.

The next step was to assist in planning the installation of these machines and to continually encourage the use of some type of flavor-removal machine. We never recommended one make of machine over another. I think evidence of this fact is that the various makes of flavor-removal machines are about equally distributed throughout North Carolina.

The final step, which is still in progress, is to assist the plants when they have trouble. One problem developed because of a change in boiler compounds. Another concerned the freezing point. Still others were related to the keeping quality. One or two plants reported that steam- and vacuum-treated milk did not have the shelf-life it should have. A visit to the plant and about three days of good sanitizing procedures showed that the fault was not due to the vacuum and steam treatment.

This is a long story to answer the first portion of the question, but we believe this approach has been successful. In 1959, 85% of the fluid milk processed in North Carolina was through some type of flavor-removal equipment. This has been done since 1955. We know that our farmers are happier, and we seldom hear complaints from them about plants rejecting milk. The processors are happier. Fluid milk sales in North Carolina have increased four times as much as that of the United States. The equipment companies have sold thousands of dollars worth of equipment and have been stimulated to think of other possible sales.

In answer to the second portion of the question regarding special methods that have proved practical in dealing with organized groups in the dairy and food industry, I am again going to cite an example of an organization that was founded in 1944, known as the North Carolina Dairy Foundation. On the back of the little folder that was sent out to all dairy farmers, processors, and supply men this year and in previous years, we find these words:

"The Dairy Foundation was organized as a corporation in 1944. This nonprofit organization of dairy producers, dairy supplymen, and

processors raises funds for supplementing and supporting dairy research, teaching, and extension work at North Carolina State College.

"Purpose: Sixteen years ago, a group of dairy leaders recognized the need for know-how and the potential of an expanded dairy industry in North Carolina. By supporting State College and working together, this Dairy Foundation is helping meet the challenge of expansion.

"Money: In seeking some plan whereby everyone could participate in this fund-raising effort, the group adopted the June Dairy Month Educational Plan. Under this program, dairy farmers contribute 5¢ per hundredweight on the amount of milk sold during June. Milk processors match this with an additional 5¢ per hundredweight on milk and ½¢ per gallon on ice cream.

"The significant growth of the dairy industry in North Carolina has paralleled the beginning and development of the Dairy Foundation."

Since 1944 this organization has supplemented research, teaching, and extension at North Carolina State College in the amount of $233,997. It has had a total income of $535,027. This does not include 1960, but we hope that the income this year will be about $35,000 to $40,000.

As you can see, this is considerable money, but its benefits go much further. As the Director of our Foundations, L. L. Ray, so aptly stated—"It's not the money we're after—it's the interest and support. We could have raised the money through the legislature but it would not have stimulated the interest or developed the feeling of ownership. As it is now each group—the producers, the processors, and supply men—have a feeling of belonging and that they are a part of North Carolina State College."

This is the philosophy we have followed in all of our North Carolina programs, whether it be dairy, fertilizer, eggs, fruits, or what have you. We always put a man from industry to head the committee. Why? Because those people know their needs. Our job is to furnish the information. If we help them solve their problems then they will be glad to furnish the capital to work on future anticipated problems and above all we will keep their companionship. As long as you can keep a speaking acquaintance with a person you can advise him.

As a result of this close relationship between producers, processors, and supply men, many things have been done either directly or indirectly. Here is a list of a few of the activities of the North Carolina Dairy Foundation and Industry organizations:

1. A scholarship program sponsored by the North Carolina Dairy Products Association furnishing two $500 freshman, two $300 sophomore, and two $200 junior scholarships in dairy manufacturing each year.
2. A Tarheel supplymen's professorship.
3. Six graduate research assistantships furnished by Dairy Foundation. Two more are anticipated in 1960.
4. Dairy Foundation has supported these activities:
   (a) Furnished funds for salaries for 1 yr. for new persons relieving one who is retiring, in order that there will be no lag in the program.
   (b) Furnished money to supplement salaries of persons to establish a regional DHIA IBM record processing center.
   (c) Furnished funds to supplement salaries of professors to such a degree as to maintain a highly qualified staff.
   (d) Furnished funds for special visiting lecturer programs to bring to campus outstanding scientists in the field of dairying, bacteriology, and related areas.
   (e) Furnished a portion of funds for a consumer marketing specialist.
   (f) Furnished funds not regulated by the General Assembly which meets every 2 yr. Sometimes problems arise which can not be anticipated. Thus, another function of this source of revenue is that it provides flexibility to the dairy program.

In summary, we can state that in working with industry in any way we must do these things:

1. Create an atmosphere in which to serve.
2. Be qualified to assist by keeping abreast of their problems.
3. Stick to the facts and don't worry too much about hurting the feelings of a segment that opposes a controversial issue.
4. Make sure there is some economic significance attached to the problem.
5. Keep your industry informed of how their money is being used.
6. Never refuse to give assistance when called!

COORDINATED STATE-WIDE PROGRAMS FOR PRODUCER'S MILK QUALITY IMPROVEMENT

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After many decades of quality improvement work, obtaining consistently high quality milk from all patrons continues to be one of the most bothersome problems for most dairy plant operators. This is true for all of the dairy industries, market milk as well as the manufactured milk industries.

E. Wallenfeldt

In the market milk industry, particularly, bacterial plate counts have been reduced markedly; but other phases of quality have regressed in some areas. In fact, with the adoption of more effective cooling techniques, some dairymen seem to have become less meticulous about real sanitation methods such as cleanliness in milking, cleaning, and sanitizing of milking machines, utensils, etc.

Surveys on many markets show that much attention is needed towards the elimination of common off-flavors. Recent happenings have emphatically called attention to the need for more effective programs on prevention of contamination with chemical adulterants such as antibiotics, insecticides, and weed killers. In many areas, the seriousness of the mastitis problem has become more acute.

These are but a few of the illustrations that can be cited on problems of quality in raw milk.

COORDINATED-INTERAGENCY PROGRAMS ON MILK QUALITY IMPROVEMENT

A number of states have worked on integrated quality improvement programs for many years. In Wisconsin, these involve many departments within the College of Agriculture, as well as other agencies such as the State Department of Agriculture, the State Board of Health, the State Board for Vocational and Adult Education, dairy products associations, producers' associations, and others.

ESSENTIALS FOR SUCCESSFUL STATE-WIDE QUALITY PROGRAMS

Experience indicates that the following are essential for consistent success with state-wide quality improvement programs:

1. Favorable public opinion.
2. Effective industry quality programs.
3. A comprehensive quality code with consistently effective regulatory enforcement.
4. Continual educational activities supporting quality improvement at every opportunity.

DEVELOPING AND MAINTAINING FAVORABLE PUBLIC OPINION SUPPORTING QUALITY IMPROVEMENT

The problems involved in developing and maintaining favorable public opinion can not be solved by any group alone. They require constant cooperation and understanding of all the key individuals in all of the agencies concerned, such as: (a) district extension leaders, (b) agricultural and home economics extension specialists, (c) county extension agents (agricultural, home economics, 4-H club, and farm and home development agents), (d) other educational agencies such as the State Board for Vocational and Adult Education and the State Department of Public Instruction, (including agricultural and home economics high school teachers and rural grade school teachers), (e) regulatory agencies like the State Department of Agriculture, the State Board of Health, city health departments, etc., (f) practicing veterinarians and other local professional leaders, (g) trade associations, and (h) service clubs and local business people who come in contact with dairymen.

The cooperation of these people can usually be obtained by encouraging them to participate on committees and accept specific assignments. They can help by presenting certain phases of the problem to their associates, and by arranging with their groups for programs which involve activities related to quality improvement.

The responsibilities of the dairy manufacturing specialists have been to furnish both technical and practical information to the participating individuals and groups, to help in the development of sound dairy quality programs by the producers' associations, and the dairy processors, and to carry out educational programs to convince people of the value of quality improvement.

Experience has shown that consistently effective quality programs can not be maintained on a state-wide basis without the development and maintenance of the kind of public opinion that is favorable. This has proven essential for the effectiveness of both industry and regulatory enforcement programs.

EFFECTIVE INDUSTRY QUALITY PROGRAMS

Consistently high quality is not likely to be obtained without effective individual plant quality programs. These must involve all patrons. The following features of such plant programs are important:

1. Quality-conscious management dedicated to quality improvement.
2. Good plant sanitation practices and good plant housekeeping.
3. Rigid intake inspection of all milk or cream received and refusal to buy any that is unfit.
4. Good laboratory facilities and frequent quality testing of all milk and cream received.
5. Good records of defects found by intake inspection, quality testing, and field work.
6. Effective field work by competent fieldmen.
7. Efficient washing and sanitizing of all cans and transport tanks and daily inspection of cleaning and sanitizing procedures.
8. Providing suitable sanitation equipment and supplies for patrons at reasonable cost to them.
9. Payment to producers for milk and cream on a quality basis.
10. Organized programs for training and supervision of all haulers.
11. Programs for aiding producers in the improvement of quality production facilities, such as improvement of milk houses, cow barns, and cow yards.
12. Good public relations programs with the general public, producers, plant employees, regulatory agencies, educational agencies, and industry associations, and cooperation with all groups working on quality improvement.
13. Programs to help in guiding unsatisfactory milk producers away from dairying into other lines of work where incurable lack of sanitation-consciousness will not be as great a liability as it is in the dairy industry.

All of these are helpful in quality improvement, but inspection of all milk purchased, quality testing, good quality records, effective fieldwork, and the relentless discouragement of all insanitary production are absolutely essential for success in quality improvement.

EFFECTIVE REGULATORY PROGRAMS

Where the educational programs for comprehensive understanding of quality improvement have been successfully carried out, sound legislation should follow without too much difficulty. Such legislation should provide for an effective state-wide quality code with adequate enforcement.

In Wisconsin, a set of minimum standards or a comprehensive code of quality conduct was developed and legally put into effect after 10 yr. of intensive educational work on quality. This was developed by a large committee representing all principal producer interests, the six major branches of the dairy industry, and representatives of the public agencies involved. These were the State Department of Agriculture, the State Board of Health, the State Board for Vocational and Adult Education, and the College of Agriculture. Legislative action at that time was not required, because a previous legislature had passed an enabling act which authorized the establishment of a comprehensive milk sanitation regulation by administrative order.

Before the enactment of this regulation, more than 6,000 meetings had been held at district schoolhouses, where quality improvement and the necessity for more rigid standards had been discussed. County-wide meetings with plant operators, farm leaders, and farmers had been held a number of times in all of the counties. Following the enactment of the regulation, meetings were again held for all farm leaders, key personnel of the plants, milk haulers, and producers in all of the counties. There were more than 2,000 such meetings, with total attendance of more than 120,000 people, in the period during which this code was being put into effect.

CONTINUAL EDUCATIONAL ACTIVITIES SUPPORTING QUALITY IMPROVEMENT AT EVERY OPPORTUNITY

Continuation of these coordinated extension activities on quality improvement seem essential to keep quality from regressing. Changes in personnel on farms and in plants are continual. Likewise, the new people entering related educational work, such as general agricultural extension and teaching, are likely to take quality for granted and overlook the necessity of persistent follow-up if these coordinated quality programs are relaxed.

SURVEY ANALYSIS OF PROBLEMS IN DAIRY PRODUCTS MANUFACTURING

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The Smith-Lever Act is responsible for the development of the Extension Service. It was enacted in 1914. The outline of the responsibility of the Extension Service gives the following: "To diffuse among the people of the United States useful and practical information on agriculture and home economics and to encourage the adoption of the same."

C. B. Reeves

In the past, the work of the Extension Service has been largely information to the agricultural interests. The Extension Service has an obligation, however, to all the people. Marketing specialists and processing controls are necessary for the best conditions of the agricultural economy. By encouraging the sale of dairy products, we improve agriculture and thereby improve the dairy industry. No phase of our national economy has progressed as rapidly as agriculture during the last 20 yr.

WHY DO PEOPLE DRINK MILK?

It is a well known fact that people consume large quantities of dairy products, but it has sometimes been vague as to why the public prefers or accepts some products over others. We are reasonably sure that people do not drink milk because it is healthy for them or because it contains all the necessary vitamins or is the most nearly perfect food but, rather, because they like it. Our educational efforts have helped, but without an appreciation of the fine flavors of milk and dairy products by children long before they understand the importance of nutrition, we would not have the dairy industry in its present high state of development.

NEED FOR QUALITY RECOGNIZED

To improve the quality of the dairy products for the consumer, the Extension Service at Clemson Agricultural College added to its program a dairy technologist. Dairying in this state is one of the smaller enterprises as far as income is concerned. The history of milk consumption has been an unsteady, fluctuating process because of insufficient supplies, varying quality standards, and inadequate educational facilities. It would be well to remember that the educational level in the southeastern part of the United States is below the average. In South Carolina, especially, certain deficiencies are evident. To obtain a teacher's certificate no course in nutrition is required. The 3 R's have been practically the only intent of maintaining the educational program, which until a few years ago included only 11 grades.

CLEMSON SURVEYS

To improve the dairy industry in South Carolina an attempt was made to improve the quality of the finished bottle of milk and of other dairy products on the market. Surveys of dairy products during a 4-yr. period have revealed some rather surprising results. The surveys have been of two kinds, one of dairy products and the other of markets. An outline of the two surveys was prepared and distributed to the dairy plants in the state.

DAIRY PRODUCT SURVEY

A dairy products survey was first conducted during October of 1956 and has been made on a more or less continuous basis ever since. The intent of a product survey is to obtain milk as a customer would on the open market. In most cases the samples in any one survey are obtained in as near one market location as is possible. The samples are brought to Clemson College under refrigeration, and tested for the following, insofar as is possible: Flavor, coliform, standard plate count, psychrophilic bacteria, phosphatase, freezing point determination, fullness of container, percentage fat, percentage solids-not-fat, fatty acid degree, acidity, and homogenization efficiency. Seldom are all of these tests done, because facilities are not always available. When a special problem arises, an effort is made to analyze certain samples in more detail.

This is not an attempt to obtain a representative sample from any particular company. It is, however, a sample as one customer might obtain it. The bacterial and coliform counts, or any other tests, are not considered to be official or are not to be used in any way to indicate the superiority of any one brand of milk over another. It is designed to aid in improving the general quality of milk.

The flavor score and standards are taken from the American Dairy Science Association's Student Scoring Standards for milk quality.

They are as follows:

Score	Grade	Criticism or defects
40	Excellent	No criticism
37–40	Good	Lacking special high flavor, flat, very slight feed, slight cooked
34–37	Fair	Cooked, feed, salty, slight cowy, slight oxidized, slight unclean
25–34	Poor	Strong feed, weedy, bitter, strong musty, cowy, oxidized, slight rancid, or unclean
Below 25	Bad	Rancid, strong cowy, high acid

Some objection has been made to the use of the word criticism in the case of milks which are of good or excellent quality. It would be more accurate to say that when scores of 37 or higher are found it should be considered not as a criticism but as a flavor profile. Scores of 37 and above, we feel, are excellent for commercial milks. They should result in consumer acceptance. Scores between 34 and 37 should definitely be improved. Scores of 34 or less, we feel, will have a great probability of encountering consumer resistance.

The best quality control program is never good enough to be considered perfect and it is, therefore, the desire and intent of these surveys to aid the program that each dairy maintains. It is not to be a substitute in any way for other control programs. It is designed to give the consumers throughout the entire market area better milk.

It was soon found that the Dairy Products Survey did not give us as much improvement as we expected. As a result, in September of 1958 a market survey was started which it was hoped would improve, to a marked extent, the products of a particular dairy operation.

MARKET SURVEY

A Market Survey is a survey of one particular dairy's operation and samples are obtained in the regular market channels of that dairy. It is necessary in this type survey to obtain, from every dairy, a code of its bottling dates so that the age of each sample is known. It is the intent to obtain samples which are as inferior as it is possible to obtain in any market.

At the time of collection, the temperature of the samples is recorded. A note is made of the products in each particular location and the condition of the display. Also, notes are made of the competitors in each particular location. The samples are obtained in large and in small stores. It is believed that, in general, the bottled milk is good as it leaves the plants, but delays in sales, delivery, and distribution cause losses to plants which should be corrected. This was the objective of the Market Survey.

The samples obtained were taken to the dairy plant where they were processed and judged in the presence of processing, distributing, and sales personnel. A list of the sources and conditions found is compared with the products.

RESULTS

Two surveys have resulted in much information. It is being transferred to IBM cards and tabulated, so that further analysis and comparisons can be made. Only a part of the data obtained has been processed. Of 423 samples of homogenized milk from the Dairy Products Survey, the flavor scores have indicated a large percentage of the milk within the classification Good. However, those scores which are below 37 give us considerable concern. Of the 423 samples, 143 have scored below 37, which is almost 34%. It is our feeling that consumers should have all milk scoring 37 or above. A very discerning child may object to the feed flavors which may be present in the 37-score milk. Very slight oxidized flavor falls in this category.

When anything but the best is served there is danger of prejudicing individuals so that their permanent milk drinking habits are affected. Until every bottle of milk available to the customer has a score of 37 or above we will find people who do not like to drink milk. We must remember the formative period in a person's likes or dislikes usually occurs before the age of six.

AGE OF MARKET MILK VARIES

With a large number of samples scoring below 37 the Market Survey was analyzed and some startling information was obtained. The age of milk on the market varies a great deal. Since the Market Survey was an attempt to find poor quality milk, it does not represent a true picture of a plant operation. Therefore, the average age of the product found in a Market Survey does not in any way represent the average age of milk on the market. It is assumed that milk should keep for possibly a week in good condition, but when 33 of 43 samples were over 1 wk. of age at the time of sampling, which does not include time in a home refrigerator, it is a pathetic situation. When milk is still available after ten days—and 24 were found after that date—it is even more deplorable. It is hard to realize that in the Market Survey 13 samples of milk out of 43 samples on the market were more than 2 wk. of age. Three samples were 3 wk. or more in age.

PROTECTION MUST COME FROM DAIRIES

Any dairy allowing poor quality milk to remain on the market will not stay in business

long. The consumer must be protected and this should not come about by legislation, as is true in some states, but by the industry itself. A dairy must police its own premises and its own delivery systems, to maintain a high quality product.

It is evident, from the Market Survey and from comparisons of different dairies and their results, why some dairies continually complain of their inconsiderate competitors. It seems that they spend much of their time complaining about the bad practices of their competitors and fail to realize they are hurting the quality of their milk products.

OTHER MILK PRODUCTS SURVEYS

Comparable surveys have been made on Cottage Cheese, Buttermilk, Skimmilk, and Chocolate milk. In the case of one dairy, no sample of milk, cream, or other product was found to be over five days of age in the grocery store. Usually, of course, it is one day old by the time the grocery store obtains a supply.

Companies that maintain a good quality control and a good delivery system will find competition much less difficult than those who have no interest or concern for the quality of their products. A quality control program must start with the cow and end at the consumers' table. When it stops in the cooler of the processing plant it falls short of achieving the desired objectives.

Resurveys on companies who have developed programs of control all through the line have shown the Market Survey to be, in some instances, highly successful. In other instances the dairies continued to have difficulty.

A METHOD FOR CALCULATING THE WEIGHT PER GALLON OF FLUID DAIRY PRODUCTS

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The manufacturer of dairy products is frequently faced with the necessity of knowing the relationship between gallons and pounds of fluid dairy products. The values given in the literature for the weight per gallon of some dairy products vary considerably and it is difficult to determine the standard which should be used for each product. The Department of Commerce (1) gives the weight per gallon of 40% cream at 50° F. as 8.38 lb., whereas the USDA (2) lists 8.39 lb., Frandsen *et al.* (4) gives 8.3 lb., and Lampert (7) 8.37 lb. Other values used by the industry range from 8.25 to 8.40 lb. per gallon for 40% cream. The original measurements of weight per gallon of cream, given in reference (3), were made by Bearce (1) in 1914. On page 262 Bearce (1) states . . . "There was in many cases a considerable amount of separation and churning of fat so that granules of butter were collected on the neck and the cap of the bottle in which the sample had been kept." Thus, these so-called standard density values were determined in a period when the full significance of the physical state of the fat as a variable factor in milk density was not understood. Bearce (1) did observe anomalous behavior in rate of expansion values of cream, and suspected that the physical condition of the fat might be the underlying cause of the discrepancies.

There is considerable variation in the specific gravity of milk fat, as given in the literature. Richmond (10) gives a specific gravity of milk fat of 0.93 at 15° C. (59° F.) and Hunziker (5) states that at 60° F. it is approximately 0.93. According to Koestler (6) the specific gravity of milk fat ranges from 0.9355 to 0.9448 at 15° C. (59° F.). Davies (2) gives a range of 0.936 to 0.940 at the same temperature, whereas Ling (8) cites the range as being 0.930 to 0.946 at 15° C. (59° F.).

Sharp and Hart (11) point out that the previous temperature history of the milk influences its specific gravity at 15° C. (59° F.) if this constant is measured immediately on reaching this temperature. There is a lag in the melting and solidification of the fat with changes in temperature, the magnitude of the effect increasing with the fat content. These authors state that a more unsatisfactory temperature than 15° C. could not be found. Lack of appreciation of the importance of this lag factor may account for the reported variations and ranges for the specific gravity of milk fat.

Sommer (12), Frandsen (4), and others have presented formulas for calculating the weight per gallon of ice cream mix. Pien and Maurice (9) have published formulas for calculating the weight per liter of milk, skimmilk, cream, and condensed whole milk. Generally, the weight per gallon may be computed from these formulas with a fair degree of accuracy within certain limits of composition. However, when existing formulas for ice cream mix are applied to other fluid dairy products, variations from actual weight can be of such

magnitude that the calculations are of little value in ascertaining weight per gallon. The need for a simple and accurate method of calculating the weight per given volume of fluid dairy products has been recognized by the industry.

DETERMINATIONS OF SPECIFIC GRAVITY OF PRODUCT COMPONENTS

Specific gravity was determined by weighing a gallon of product, using a Bureau of Standards certified pycnometer (1-gal. capacity) and a balance sensitive to 1 g.

Milk fat. The pycnometer was held in a room at 36° F. and melted fat added in increments of about 300 ml. Each portion of melted fat was allowed to solidify and age for 4 hr.

TABLE 1

Specific gravity of butterfat and density of water at various temperatures

Temperature ° F.	Butterfat [a] Sp. G. at $\frac{T}{40}$	Water [b] density
32	.966	.99987
34	.964	.99993
36	.962	.99997
38	.960	1.00000
40	.958	1.00000
42	.956	.99998
44	.954	.99994
46	.952	.99989
48	.950	.99982
50	.948	.99973
52	.946	.99962
54	.944	.99950
56	.942	.99937
58	.940	.99921
60	.938	.99903
62	.936	.99885
64	.934	.99866
66	.932	.99845
68	.930	.99823
70	.928	.9980
75	.924	.9974
80	.921	.9966
85	.918	.9958
90	.914	.9950
95	.910	.9941
100	.907	.9931
105	.905	.9920
110	.903	.9909
115	.901	.9898
120	.899	.9886
125	.897	.9873
130	.896	.9860
135	.894	.9846
140	.892	.9832
145	.890	.9818
150	.888	.9803
155	.887	.9788
160	.885	.9772
165	.883	.9755

[a] Obtained from weighings at several temperatures.

[b] From Bureau of Standards—Standard Density Tables—Circ. 19.

before the next increment was added. The reason for adding the fat in small increments was to avoid fissures which normally develop during solidification of fat. This procedure was followed until the pycnometer was filled with milk fat at 36° F. Since only two portions of fat were added each day, about a week was required to completely fill the pycnometer. The weight of fat was determined and the specific gravity of milk fat based on the actual weight was 0.962 at 36° F. The storage temperature was elevated to 40° F. and the product held for seven days, at which time the fat above the surface of the pycnometer was removed and the product reweighed. The specific gravity at this temperature was found to be 0.958. This procedure was repeated and the specific gravity determined at 50, 60, and 70° F. Additional specific gravity determinations were made on melted milk fat at various temperatures up to 165° F. These results are given in Table 1.

The observed weights per gallon of 40% cream were used to demonstrate the change in specific gravity of milk fat during solidification. It was found that the greatest increase in specific gravity occurred during the first half-hour after cooling to 50° F. or lower. Solidification of the fat appears to be complete and the specific gravity at a maximum after 4 hr. of holding at the indicated storage temperature. The difference in the weight per gallon of 40% cream, at temperatures between 32 and 50° F., held for various lengths of time, is used in calculating the change in specific gravity of the milk fat. For example, the weight of 40% cream, aged for 4 hr. or longer at 40° F., is 8.373 lb. per gallon, whereas the same cream weighs 8.330 lb. per gallon immediately upon cooling to 40° F. This is a difference of 0.043 lb. per gallon for 40% cream or 0.108 (0.043 × 2.5) per gallon for milk fat. Thus, with a weight of 7.995 lb. per gallon for aged milk fat at 40° F. (corresponding to a specific gravity of 0.958), the milk fat immediately upon cooling would have a weight of 7.887 (7.995–0.108) lb. per gallon, which corresponds to a specific gravity of 0.945. These data are given in Table 2.

Solids-not-fat. The specific gravity of solids-not-fat was determined by calculating the volume displacement in toluene of a given weight of air-free sugar and milk solids-not-fat. Sugar has a specific gravity of 1.56 and milk solids-not-fat, 1.47. Weighings were made of sugar solutions, fluid and condensed skimmilks, and sweetened condensed skimmilk. From these weights it was found that although sugar and milk solids-not-fat are of slightly different specific gravities, products containing the same quantity of solids-not-fat had essentially the same weight per gallon. For example, the following products were found to have the same weight of 9.40 lb. per gallon at 40° F: (a) solution containing 30%

TABLE 2

Specific gravity of butterfat at $\frac{T}{40}$

(For products cooled to and held at the indicated temperature following pasteurization)

Storage temperature	4 hr. and longer	2.0 hr.	1.0 hr.	0.5 hr.	0 hr.
(° F.)			(Sp. G.)		
32	.966	.965	.963	.960	.953
34	.964	.963	.961	.958	.951
36	.962	.961	.959	.956	.949
38	.960	.959	.957	.954	.947
40	.958	.957	.955	.952	.945
42	.956	.955	.953	.950	.943
44	.954	.953	.951	.948	.941
46	.952	.951	.949	.946	.939
48	.950	.949	.947	.944	.937
50	.948	.947	.945	.942	.935

sugar, (b) 30% condensed skimmilk, (c) blend containing 15% sugar and 15% milk solids-not-fat. Other nonfat solids, such as stabilizers, cocoa, etc., act in a similar manner.

Water. Specific gravity values for water were obtained at several different temperatures, using the pycnometer. These values were the same as those given in standard Density and Volumetric Tables (3). The specific gravity of water at various temperatures from 32 to 165° F. is presented in Table 1.

DEVELOPMENT OF THE METHOD USED FOR CALCULATING THE WEIGHT PER GALLON OF FLUID DAIRY PRODUCTS

In theory, the sum of the values, obtained by multiplying the density of the ingredient by the percentage of ingredient should equal the density of the blend. However, this is true only when the ingredients are immiscible. For example, a blend of 50 parts of milk fat and 50 parts of water has a specific gravity equal to the average of that for fat and water. On the other hand, the specific gravity of a blend of 50 parts of sugar and 50 parts of water is not equal to the average of 1.28 for that for sugar and water, but rather 1.2295. Thus, in devising a formula which would be suitable for application to cover all fluid dairy products, it was necessary to calculate factors for a complete range of solids-not-fat. A typical calculation for

TABLE 3

Factors for total solids-not-fat [a]

(M.S.N.F., nonfat chocolate solids, cane sugar, corn sugar solids, stabilizer, vanilla powder, etc.)

%	Factor	%	Factor	%	Factor	%	Factor
1	1.2120	22	1.4086	43	1.4465	64	1.4848
2	1.3007	23	1.4105	44	1.4483	65	1.4867
3	1.3311	24	1.4124	45	1.4501	66	1.4885
4	1.3470	25	1.4142	46	1.4519	67	1.4904
5	1.3570	26	1.4161	47	1.4537	68	1.4922
6	1.3642	27	1.4179	48	1.4555	69	1.4941
7	1.3697	28	1.4197	49	1.4573	70	1.4960
8	1.3743	29	1.4215	50	1.4591	71	1.4978
9	1.3781	30	1.4233	51	1.4610	72	1.4997
10	1.3814	31	1.4251	52	1.4628	73	1.5016
11	1.3844	32	1.4269	53	1.4646	74	1.5034
12	1.3872	33	1.4286	54	1.4664	75	1.5053
13	1.3897	34	1.4304	55	1.4682	76	1.5072
14	1.3921	35	1.4322	56	1.4701	77	1.5090
15	1.3944	36	1.4340	57	1.4719	78	1.5109
16	1.3966	37	1.4358	58	1.4737	79	1.5128
17	1.3988	38	1.4376	59	1.4756	80	1.5146
18	1.4008	39	1.4393	60	1.4774	81	1.5165
19	1.4028	40	1.4411	61	1.4793	82	1.5184
20	1.4048	41	1.4429	62	1.4811	83	1.5202
21	1.4067	42	1.4447	63	1.4830	84	1.5221

[a] Calculated from Bureau of Standards—Standard Density Tables—Circ. 19—6th ed.

the factors, as presented in Table 3, is as follows: Example: 20% factor

100 ×	density of 20% solution (3)	108.0959
80	(Water phase of desired 20% solution) × (density of water)	80.0000
20	TSNF × factor desired = or factor for 20% TSNF (Table 3) =	28.0959 / 1.4048

With these factors, it is possible to calculate the specific gravity of blends of solids-not-fat.

Since fat is not soluble in water, the true percentage of total solids-not-fat in a product, as related to density, is a function of the moisture content. Thus, in a product containing 20% fat, 24% TSNF, + 56% water the percentage of TSNF in the nonfat phase is 30%.

$$\frac{\%\ \text{TSNF} \times 100}{\%\ \text{TSNF} + \%\ \text{water}} \text{ or } \frac{24 \times 100}{24 + 56} = 30\%$$

The factor of 1.4233 corresponding to 30% TSNF would be used for calculation purposes.

The density of milk fat increases with time, after pasteurization and cooling. Values for the density of milk fat at temperatures from 32 to 50° F., and after holding at this temperature for 0.5, 1, 2, and over 4 hr., are given in Table 2.

CALCULATION OF WEIGHT PER GALLON OF FLUID DAIRY PRODUCTS

The general formula and a typical calculation of weight per gallon are given in Table 4.

The weight per gallon at 40° F. is obtained as follows:

1. Percentage of fat is multiplied by 0.958 (density of milk fat at 40° F.).
2. The sum of the percentages of all solids-not-fat is multiplied by the factor from Table 3 corresponding to the solids-not-fat in the nonfat phase; or factor corresponding to: $\frac{\%\ \text{TSNF} \times 100}{\%\ \text{TSNF} + \%\ \text{water}}$
3. Percentage of moisture is multiplied by 1.000 (density of water at 40° F.).

TABLE 4

Formula and example for calculating weight per gallon of fluid dairy products

$$W = \frac{A + B + C}{100} \times D$$

Where:

W = Weight in pounds per gallon

A = Per cent fat × specific gravity of fat at given temperature (Table 1 or 2)

B = Per cent total solids-not-fat × factor for solids corresponding to $\frac{\%\ \text{TSNF} \times 100}{\%\ \text{TSNF} + \%\ \text{Water}}$ (Table 3)

C = Per cent water × density of water at given temperature (Table 1)

D = Weight of 1 gal. of water at 40° F. or 8.345 lb.

Typical calculation of weight per gallon

Product: Ice Cream Mix—held 4 hr. or longer at 40° F.

Composition *(%)*:

11.2 Butterfat	
11.0 MSNF	
12.0 Sugar	
4.1 Corn syrup solids	} 27.3% Total solids-not-fat
0.2 Stabilizer	
38.5 Total solids	

$$W = \frac{A + B + C}{100} \times D$$

A = 11.2 × .958	Table I =	10.7296
B = 27.3 × 1.4246[a]	Table III =	38.8916
C = 61.5 × 1.0	Table I =	61.50000
A + B + C	=	111.1212

D = 8.345

$$W = \frac{111.1212}{100} \times 8.345 = 9.27 \text{ lb/gal.}$$

[a] 1.4246 is the factor for solids-not-fat corresponding to 30.74% total solids-not-fat in the water phase of the mix $\frac{(27.3 \times 100)}{(27.3 \times 61.5)} = 30.74$.

TABLE 5

Weight of milk products and ice cream mix ingredients at 40° F. held 4 hr. or longer after pasteurization

Product	Fat	M.S.N.F.	Sugar solids	Total solids	Weight
	(%)				(lb/gal)
Water	....	.....	.....	0.00	8.345
Skim	0.06	8.84	.....	8.90	8.62
Milk	3.0	8.40	.....	11.40	8.60
	3.5	8.60	.....	12.10	8.60
	4.0	8.70	.....	12.70	8.61
	4.5	8.90	.....	13.40	8.61
	5.0	9.00	.....	14.00	8.61
Half & half	8.0	12.00	.....	20.00	8.71
	10.0	10.00	.....	20.00	8.63
Milk-cream blend	10.0	8.01	.....	18.01	8.56
	12.0	7.83	.....	19.83	8.55
	14.0	7.65	.....	21.65	8.54
	16.0	7.47	.....	23.47	8.52
Cream	18.0	7.30	.....	25.30	8.51
	20.0	7.12	.....	27.12	8.50
	25.0	6.68	.....	31.68	8.47
	30.0	6.23	.....	36.23	8.44
	35.0	5.78	.....	40.78	8.40
	40.0	5.34	.....	45.34	8.37
	45.0	4.90	.....	49.90	8.34
	50.0	4.45	.....	54.45	8.31
Condensed	12.0	28.00	.....	40.00	9.30
	8.2	21.80	43.0	73.00	11.00
	0.2	29.80	.....	30.00	9.40
	0.2	31.80	.....	32.00	9.48
	0.2	29.80	40.0	70.00	11.23
White mix	4.10	14.0	16.0	34.30	9.40
	10.15	10.0	15.85	36.25	9.23
	10.15	11.0	17.20	38.68	9.32
	11.15	11.0	16.30	38.78	9.29
	12.20	10.5	15.50	38.50	9.23
	12.20	11.0	16.50	40.00	9.29
	14.00	10.0	15.70	40.00	9.22
	16.00	10.0	15.70	42.00	9.21
Chocolate mix	4.10	13.5	17.00	37.00	9.51
	10.10	8.0	18.40	38.81	9.33
	11.10	9.0	18.50	41.42	9.39
	12.10	9.5	19.50	44.00	9.46
	14.00	9.5	18.65	45.25	9.43
	16.00	8.0	10.00	46.00	9.37
Ice base	0.0	0.0	36.00	36.50	9.67
Sherbet base	1.2	3.55	31.25	36.60	9.62
Fudgsicle	1.0	11.68	18.80	35.65	9.63
Liquid cane sugar at 70° F.		.....	67.00	67.00	11.08
Corn syrup at 110° F.		.....	.....	81.00	11.82

Fat includes butterfat, cocoa fat, egg-yolk fat, emulsifier, etc.

In addition to the ingredients listed, total solids includes nonfat cocoa solids, nonfat egg-yolk solids, stabilizer, etc.

4. The sum of these values is divided by 100 and multiplied by the weight of a gallon of water at 40° F. (8.345 lb.) to obtain pounds per gallon.

Calculated weights of milk products and ice cream mix ingredients at 40° F. are presented in Table 5.

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MATHEMATICAL TECHNIQUES FOR EVALUATING LABOR UTILIZATION IN DAIRY PLANT OPERATIONS[1]

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Maintaining the proper balance between labor and equipment costs is a major problem of management. Equipment costs are relatively easy to determine. On the other hand, it is rather difficult to measure accurately the labor costs. Equipment costs tend to be relatively fixed; whereas, some labor costs are fixed and others are highly variable. The variable nature of labor utilization, along with the high cost of labor, makes it mandatory that management have accurate and reliable methods of measuring and controlling labor costs.

This study was designed to test procedures for collecting, analyzing, and presenting labor utilization data in a simple and usable form.

It seemed desirable to go beyond the work of Gregory, Roberts, and Hader (4), and to express certain labor requirements for processing dairy products in mathematical relationships. This required the establishment of a volume-time relationship for individual products and processes in the dairy plant, so that prediction equations could be formulated in terms of variable and fixed times, and appropriate statistical tests could be performed.

METHODS

Gregory, Roberts, and Hader (5) made a comparison study in the dairy industry between work sampling and a production study and found no significant difference to exist in the results between the two techniques. Since work sampling is better accepted by employees, is not tedious to use, results are simple to analyze, and one man can study an entire plant crew simultaneously, it was used to collect information about labor utilization in a representative dairy plant.

Before the full-scale study of a commercial dairy plant was initiated, preliminary observations were made in the North Carolina State College dairy plant to investigate the feasibility of such a study and to test the usefulness of various analytical techniques. Sufficient evidence was obtained to indicate that this type of study and analysis had a place in the dairy industry in analyzing labor utilization. Therefore, a North Carolina milk plant was selected in which further work was carried out. This plant was typical of the medium to large plants in the state.

The plant selected was a one-story operation with a daily volume ranging from 12,000-18,000 gal. during the observation period. The labor force was composed of one man in receiving, three men in cold storage, three men in shipping, and 23 men in processing-packaging. The plant operated from about 3 A.M. to about 9 P.M. six days per week.

Two alternating observers, recording data at 10-min. intervals, were used to observe the operation over the entire processing day. Thus, an observer was present to record all labor used in any portion of the plant operations for a 6-wk. period covering 36 operating days.

The forms used for the collection of the data closely resembled Form 1, as used by Gregory *et al.* (4); however, in this case there was a separate form for each product, e.g., homogenized milk (½-pints, paper).

The equipment in the plant was grouped into seven clean-up categories on the basis of related use. The equipment in each category was as follows:

Category 1—HTST, homogenizer, surges and standardizer-clarifier
Category 2—Sanitary lines, storage tanks, and pumps
Category 3—Paper fillers (two small, one large)
Category 4—Half-gallon paper filler

Category 5—Glass filler
Category 6—Separator
Category 7—Receiving room equipment

Categories 8-13 included labor used in assembling and mixing the ingredients for chocolate milk, orange drink, buttermilk, half and half, flakes for flake buttermilk, and whipping cream, respectively.

General classifications were used for jobs that did not fall into the other groupings. Category 14 included plant clean-up, dump returns, and make-out of general reports, and Category 15 included making out the paper filler reports and putting empty cases on the conveyor to the filler.

These groupings were helpful in studying the fixed labor used in the plant operation, since certain categories were concerned only with specific products. Thus, it was possible to analyze independently portions of the total operation.

The labor was divided into direct time and prorated time. Under direct time, there were fixed times such as change-over of the filler, and variable time such as the actual filling operations. Prorated time included labor which could not be tied to a particular product. Under prorated time, there were fixed and variable times. An example of a fixed prorated time was the clean-up of the HTST unit; whereas, an example of a variable prorated time was the operation of that unit. The total labor necessary for the clean-up or operation of the HTST unit was prorated on a weight basis among the products that utilized this piece of equipment.

A linear model was selected to express the mathematical relationship between the two variables, volume and time. The labor input for variable time was expected to be proportional to output, since it was composed mainly of package filling time, a machine-paced operation. The data showed a linear relationship when plotted on graph paper. It seemed unnecessary to use a more complicated model for the purpose of this study. The labor input for the fixed times was expected to be relatively constant throughout the range in volume. This was shown to be true for many products. Under these circumstances, the mean would give as good estimates of the actual situation as would a regression equation.

A regression equation was used for prediction of labor requirements for selected volumes. A regression line is a kind of moving average passing through the points that determine it, which measures the relationship between two variables; in this case, volume and labor. If a regression coefficient is not significant, then the mean would be as good an estimator as the regression equation. However, although a particular classification did not show a statistically significant regression coefficient, the regression equation might still be a better basis than the mean from which to make practical application of the data.

Since the volume varied from day to day, the analysis of covariance as outlined in Snedecor (6) was used to adjust for the variation in volume. In essence, the regression equation was a labor prediction equation that was valid to use for predicting labor requirements of various volumes of the listed products. The selected volumes should fall within the range covered in the study; however, limited extrapolation would probably be satisfactory for

TABLE 1

Fifteen categories of fixed labor requirements in man-minutes and their respective ranges, means, and standard errors

Category	Description	High	Low	Mean	$8\bar{x}$
Clean-up			Man-minutes		
1	HTST, homogenizer, surges, standardizer	380	160	228	7
2	Sanitary lines, storage tanks, pumps	550	230	385	12
3	Paper fillers (two small, one large)	700	240	407	16
4	Half-gallon filler (paper)	370	30	149	9
5	Glass filler	390	190	283	9
6	Separator	140	20	87	8
7	Receiving room, receiving equipment	200	20	68	5
Products make-up					
8	Chocolate milk	390	50	136	11
9	Orange drink	180	30	103	6
10	Buttermilk	230	30	121	13
11	Half and half	90	10	44	6
12	Flakes for flake buttermilk	50	0	28	3
13	Whipping cream	50	0	23	2
General					
14	Plant clean-up, dump returns, and make-out general reports	440	150	273	11
15	Paper filler reports and putting empty cases on conveyor to filler	350	110	219	10

TABLE 2

Statistical results from the covariance and regression analyses for the variable, fixed, and delay times for the can receiving, shipping–cold storage, and total processing operations

Description	Volume in thousands		Regression equation[a]	
	High	Low	a	b
Receiving variable time[b]	44	19	34	0.00492** (0.00133)
Receiving fixed time[b]	44	19	112	−0.00149 n.s. (0.00203)
Receiving delay time[b]	44	19	215	−0.000533 n.s. (0.00232)
Shipping[c]	4.0	2.9	1,421	0.0813 n.s. (0.163)
Cold storage[c]	4.1	2.7	491	0.251** (0.101)
Shipping–cold storage delay[c]	8.0	5.8	540	−0.0278 n.s. (0.0479)
Total processing variable time[d]	108	41	556	0.0382** (0.00584)
Total processing fixed time[d]	108	41	2,913	−0.00720 n.s. (0.00695)
Total processing delay time[d]	108	41	1,741	−0.0115** (0.00436)

Number in parentheses is standard error of b.
* Significant at the 0.01 level.
** Significant at the 0.05 level.
n.s. Not statistically significant.
[a] Regression of man-minutes (y) on volume in pounds (x).
[b] Volume in pounds.
[c] Volume in cases.
[d] Volume in packages.

approximations. The standard errors of the b's, (S_b), were presented to give some indication of the reliability of the individual b values. Since the ratio of b to S_b is an ordinary "t" test, the significance tests on the regression coefficients can be readily verified.

No statistical analysis was performed on the time necessary for the supervision of the operation, since supervisory time was very consistent from day to day.

RESULTS

Fixed times. In Table 1 are the results of a 36-day study of fixed times for several categories in the plant operation. The data show the mean and the appropriate standard error for each category.

The ranges show the extremes in daily variation of labor requirements over the 36-day period. In Category 13 (make-up of whipping cream), and Category 12 (make-up flakes for flake buttermilk), the range included zero. This was not realistic, since some labor was necessary to make up these products; however, this situation was a result of the pattern of collecting data. Since the observations were taken at 10-min. intervals, it was possible for an operation that took less than 10 min. to have no labor charged to it. These instances occurred very seldom, as illustrated by the relatively small standard error for each of the categories.

The fixed times necessary to handle a particular product can be computed from certain combinations of categories. For example, Categories 1, 2, 3, 14, and 15 are relevant for homogenized milk (½-pints, paper), while Categories 2, 4, 9, 14, and 15 are appropriate for orange drink (½-gallons, paper). One may determine what fixed times will drop out in the case of removing a particular product from the product mix, or stated in another way, find what additional fixed times would be incurred from the production of a particular product. More specifically, a plant manager might consider dropping orange drink from the product mix, with the data given in Table 2. With the remainder of the products intact, the only saving in fixed labor would be to eliminate Category 9, which was the make-up of orange drink.

Estimation equations for fixed and variable times. The data in Table 2 show the following: (1) the range in volume, (2) the significance of the regression coefficient for labor used against volume, (3) the regression prediction equations, and (4) the regression coefficient (b) and the standard error of this coefficient (S_b).

The can-receiving operation received only about one-third of the milk that was used in the plant. The remainder of the milk came in by tank trucks.

The variable receiving time was composed of the labor required to dump the incoming producer milk, put the cans into the rotary can washer, remove the cans from the can washer, and push them back to the producers on a gravity conveyor. The remainder of the variable time consisted of taking the weight and a representative sample of each producer's milk. Daily and weekly differences were not important factors influencing labor inputs. However, volume was an important factor because there was a relatively constant time per pound, thus giving the volume effect in the regression analysis.

The receiving fixed time was composed of the labor required to clean up the room and equipment. It was not influenced by the amount of milk passing through the receiving equipment. This fixed time (Category 7, Table 1) showed a great deal of variation because of the individuals doing the clean-up and the thoroughness of the job performed. Neither day, week, nor volume effects were important factors influencing labor input. Probably, the negative regression equation and decreased fixed labor time were the result of more labor for variable time (dumping milk, etc.) with increase in volume. The worker performed the fixed function more efficiently or less thoroughly.

The receiving man was in delay a great deal of the time because of poor scheduling of haulers. The negative regression coefficient would seem quite logical, since the receiving man converted the delay time into variable productive time as the volume of incoming milk increased. The day, week, and volume effects were not important factors influencing delay time.

The shipping time prediction equation might be thought of as a variable time equation. Three men were required to take the cases from cold storage and put them on the power conveyor to the wholesale and retail trucks. The truck schedule was very effective in keeping the shipping employees productive. However, in the case of lapses between route trucks, the shipping employees were usually in delay. Day, week, and volume effects were not of significance in influencing the input of labor of the shipping operation. The large a value (1421) in the prediction equation was probably the result of the limited range covered in the number of cases handled, plus the fact that shipping personnel tended to slow down in speed of work when they were not being pushed to fill an order. These reasons, plus the fact that the labor force was held constant from day to day to load a very similar volume, tended to give a relatively fixed labor requirement for this operation. If the shipping operation could have been more effectively scheduled as to labor needs, a significant volume effect might have been found. That is, if extra labor had been used during the peak needs, in order to maintain an efficient operation, and this labor used elsewhere when the regular force could handle the work, it is reasonable to assume that there would have been a volume effect on the shipping labor.

The cold storage prediction equation also might be considered as a variable time equation. The cold storage operation consisted of three men taking the cases off the conveyor from the filler and stacking them in cold storage. The labor force varied according to the number of bottle fillers that were operating. This tended to give the significant volume effect as shown in the statistical results in Table 2. Both the volume and week effects influenced labor input; however, there was no evidence of a day effect. Although personnel changes could have been responsible for the significant week effect, the authors believe that this significant value may have been the result of chance in the statistical analysis.

The shipping–cold storage delay equation was established in the same manner as the shipping and cold storage equations. For each day of the 36-day study, a determination was made for the total shipping–cold storage delay time for the volume of cases on that particular day of operation. Thus, 36 values were established over the entire range of volume studies. The covariance and regression analyses were performed, giving the results in Table 2. The shipping and cold storage delay times were combined, since occasionally employees in these operations would switch back and forth as the demand for labor shifted. The volume variable was the sum of the cases handled in each operation. There was no statistical evidence of an effect of day, week, or volume on labor inputs. Again, there was a negative regression coefficient which was a result of the same basic reason as for total delay in the packaging operation, i.e., with a fairly constant labor force, the more volume a group must handle, the less will be the delay time incurred.

It should be reiterated that these negative delay coefficients were not statistically significant; however, it is believed that they reflect the actual situation rather effectively.

The processing variable time prediction equation represented a composite of all the individual product prediction equations. Prediction equations were developed for the fixed and variable times of the 22 individual products that were produced in the plant. Information analogous to that found in Table 2 was given for each product.[3]

It was statistically feasible to express the volume-time relationship for all packages in one regression equation. A determination for total variable time was computed for the total packages on a particular day of operation for each day of the 36-day study. Thus, a series of points or numerical time values were established over the range in volume covered (41,000 to 108,000 total packages). The covariance and

[3] If one wishes to obtain this information, the Ph.D. dissertation (3) may be obtained from North Carolina State College.

regression analyses were performed on these data and the results shown in Table 2. The value of the over-all prediction equation is evident, since it simplifies the computation necessary in arriving at the predicted labor needs for particular volumes processed. Volume was very effective in changing labor input; whereas, the day and week effects were not of significance.

The processing fixed time prediction equation was established by summing the categories in Table 1 for any specific day of operation, and plotting the point against the volume produced on that day. Thus, over the 36-day period the numerical fixed time values were plotted against the volume covered (41,000 to 108,000 packages). On these data, covariance and regression analyses were performed and the results shown in Table 2. The fixed time prediction equation illustrated that fixed labor needs were influenced little by changes in volume. In fact, total fixed time decreased slightly as total volume increased. This resulted from a labor force that was sufficient to handle the operation under practically any circumstances; but, when the total volume of product increased greatly, the labor crew was forced to substitute a certain amount of what had previously been fixed labor into variable labor, to take care of the longer operation of the bottle fillers. The day effect did not influence this labor input according to the statistical analysis; however, the week effect was significant at the 0.05 level, which could be a result of changes in personnel between weeks, since this study was made in the vacation season.

The processing delay time prediction equation was established by summing the delay observations in the processing-packaging operation on a specific day and plotting the value against the volume produced on that day. This gave a series of determinations over the range covered (41,000 to 108,000 packages) in the study over the 36-day period. On these data, covariance and regression analyses were performed and the results shown in Table 2. Neither the day nor week effect was significant as factors influencing the total delay time. However, volume was an effective factor, and as volume increased, total delay time decreased. This was a very logical result, since the labor force was kept consistent from day to day. Thus, if the labor crew was sufficient to handle the maximum volume produced and the labor crew was held constant, then it was very reasonable that there was more delay when the volume was relatively low.

Illustrations of the use of estimation equations. The equations that have been presented can be used in several ways by management. First, they can be used as a management control on the labor used in the plant operations. Then, they can be used for estimating the labor needs by products for cost accounting and for determining potential changes in the product mix.

The latter will not be illustrated, since it is rather voluminous, but these mathematical equations used under various plant situations for estimating labor needs can be obtained.[3]

Equations used as a management control. The equations can be used to determine the total labor needs or the labor needs by processes or operations. The following illustrates how the commercial plant can utilize the equations in Table 2 to estimate the labor needs in the processing and shipping–cold storage operations.

The labor needs are shown over the range covered in the plant study (40,000-110,000 packages). The labor used in the receiving operation was not included, since the range in volume was insufficient. Also, the supervisory time was not included, since this was a constant time over the entire plant study. However, it would be simple to make this additional calculation if it were desired (1,567 man-minutes ÷ total packages = supervisory time per package).

The confidence limits as shown by the curved lines in Figures 1, 2, and 3 were established at the 0.95 level. The true value for labor required at any particular volume lies in the range between the upper and lower limits. It might be pointed out that the sample size (days of study) will influence these confidence limits. For example, if a 50-day study had been made rather than the 36-day study, the limits probably would have been narrower. Since the square root of the sample size is used in the mechanics of making the calculation, the confidence limits are usually narrowed.

Figure 1 shows the total variable, total fixed, and total delay time required, with the compu-

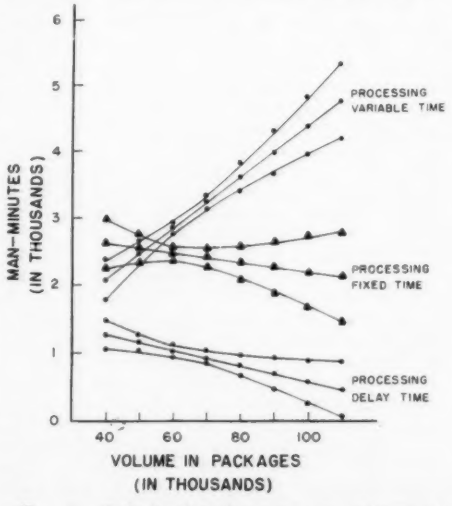

FIG. 1. Relationship between the volume of packages processed in the processing operation and the total man-minutes required, with the appropriate confidence limits.

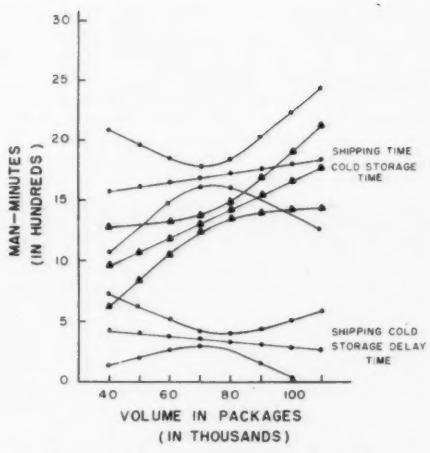

FIG. 2. Relationship between the volume of packages in the shipping–cold storage operation and the total man-minutes required, with the appropriate confidence limits.

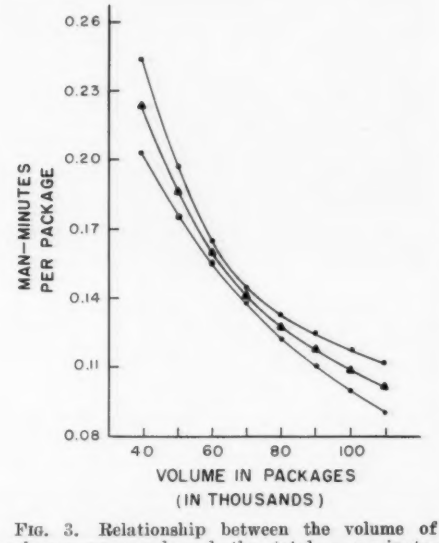

FIG. 3. Relationship between the volume of packages processed and the total man-minutes per package, with the appropriate confidence limits (summarizes Figures 1 and 2).

tations being made at each 10,000-package level. The confidence intervals were established for each of the calculated values. It should be noted that the intervals are narrowest near the mean, and swing out at the extremes in volume. Therefore, this should be taken into consideration in predicting labor needs at the extremes in volume, as more variation from the predicted values should be expected for these determinations.

Figure 2 shows the total shipping time, total cold storage time, and total shipping–cold storage time required, with the computation being made at each 10,000-package level. The confidence intervals were established for each of the calculated values. A percentage volume of half-gallons, quarts, pints, and half-pints very similar to that of the plant study was assumed. The assumed percentages in the container sizes were 15% (½-gallons, paper), 25% (quarts, paper), 15% (pints, paper), 40% (½-pints, paper) and 5% (quarts, glass). Using these percentages, the conversion of the packages into cases was made and the labor used was calculated by the use of the equations in Table 2.

Figure 3 summarizes the data in the first two figures and puts the labor used on a per package basis rather than on the total time basis. Again, computations were made at each 10,000-package level and the appropriate confidence intervals were established. In essence, Figure 3 gives an over-all view of the effect of volume in a plant operation. For example, if the production in packages per day was approximately 40,000, the man-minutes of labor used per package would be about 0.22 (upper limit 0.24 and lower limit 0.20); whereas, if the packages per day were near 110,000 the man-minute needs per package would be about 0.10 (upper limit 0.11 and lower limit 0.09). This is definitely a large saving in labor used per package as a result of an increase in volume. This savings would be even more greatly magnified if the fixed supervisory time had been included in the graph, since in the case of smaller volume production, each unit of product would have to bear a greater share of the supervisory time than would the units in the larger volume production.

Figure 3 represents the range in volume that would ordinarily be covered in the plant studied. In this operation, the labor force was held quite constant over the entire range of volume. Thus, it would not be good to extrapolate in Figures 1 and 2 to visualize the effect on per package labor used in Figure 3, since below 40,000 and above 110,000 a change in the labor force might be made, which would probably change the relationship shown in Figure 3.

Since one usually has a fairly accurate record of the plant labor used, by the time cards of the employees, daily comparisons may be made with Figure 3 to determine daily productivity of labor. If the assumption is made that the curve in Figure 3 represents a desired level of efficiency, then this curve can be used as a standard in controlling labor used. To calculate the labor used from Figure 3, it is only necessary to know the volume of all products produced on a particular day, read off the man-minutes used per unit, and then multiply the per unit value by the total units produced. For example, if 70,000 units were produced, the per unit value would be 0.142 man-minutes

and the total labor used would be 9,940 man-minutes. The lower limit (0.139 man-minutes per unit) is 9,730 man-minutes, and the upper limit (0.145 man-minutes per unit) is 10,150 man-minutes. One would expect the labor used on that particular day to fall between 9,730 and 10,150 man-minutes.

One could use an analogous procedure in determining the labor needs in the operations of Figures 1 and 2. However, in this situation, it would be necessary to have some practical breakdown of labor whereby these operating times could be separated on a daily basis.

It should be noted from Figure 3 that as the total volume increases, the labor used per unit decreases at a decreasing rate, i.e., the labor used per package was reduced substantially more from 40,000 to 50,000 packages than at higher volumes. This is in agreement with hypothetical plant studies concerning economies of scale that are found in the literature, i.e., as the total volume increases within a plant operation the amount of labor used per unit will usually decrease (1, 2).

A few statements may be in order concerning some uses of mathematical equations that were determined to describe a dairy plant operation under varying situations. One may use the equations in the estimation of the labor used at various volumes of production. Estimations by individual products or by operations can be made. Potential changes in the operation, such as the elimination of some products from the product mix or every-other-day production of certain products can be evaluated. The technique also gives the accountant fundamental labor data that may be used in showing plant costs. Labor for individual dairy products has always been information much desired by the accountant but very seldom obtained. Labor data drawn in the form of equations give the accountant a most valuable tool in his plant cost analysis. The accountant can use the curves established in Figures 1, 2, and 3 in the breakdown of plant labor, supply the proper labor cost per man-minute, and determine the labor cost by products, by processes, or operations, or for the total plant operation.

The techniques used for studying plant labor in this study are sound and practical in application. The statistical analysis and the determination of the mathematical equations must be left to individuals trained in the field. However, the finished data can be easily understood and manipulated by the dairy plant operator. Such data make available to management and accountants useful tools to aid in control of labor, decision-making, and evaluation of plant labor used.

SUMMARY

A comprehensive study of labor utilization was made in a commercial dairy plant. An attempt was made to correlate certain types of plant labor with total volume, e.g., processing variable and fixed and delay time; shipping, and cold storage time; and total time. The labor utilization data on an actual operation can serve as a guide in making comparisons with hypothetical plant studies.

The data collected on labor utilization in a commercial plant study were treated statistically. The standard errors were found for the fixed times. An analysis of covariance was carried out on the labor used in each of the individual products, testing both the variable and fixed times for differences in labor used between days and between weeks and for significance of the regression coefficients. A regression equation was computed for each operation and the standard error of b was given.

The results from the commercial plant study showed that only a few products exhibited significant day-to-day differences in labor used; whereas, weekly differences showed up in about one-third of the products. The regression coefficients were highly significant in most of the products, indicating that the labor used varied with a change in volume.

An illustration was given to demonstrate the use of the equations as a management control on the plant labor used in a commercial dairy plant. The importance of volume in an actual operating dairy plant in effecting a relatively low labor requirement per unit was pointed out. The results from the volume-labor analysis were in agreement with hypothetical plant studies concerning economies of scale that are found in the literature, i.e., as the total volume increases within a plant operation, the amount of labor used per unit will usually decrease.

Also, the use of the mathematical equations was illustrated in some plant situations to show how they might be used for determining the labor used.

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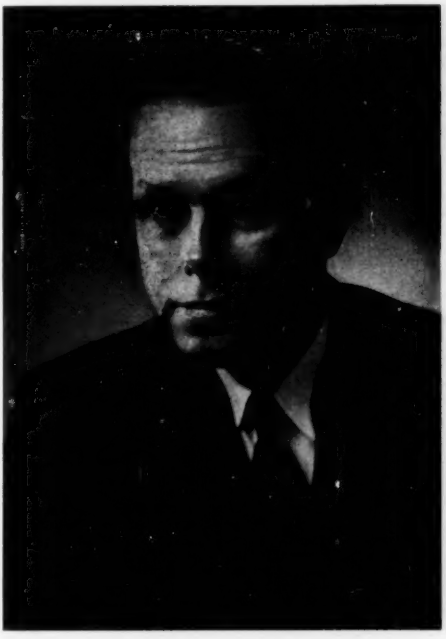

The University of Wisconsin is honored to be host to the 56th annual meeting of the American Dairy Science Association June 11 to 14, 1961.

It is always a pleasure to have visitors to our campus. Because dairying is such an important part of Wisconsin, we particularly welcome the valuable information and stimulating discussions a group such as yours will bring us.

Since you last met here in 1953, we have added several new buildings and facilities. I think you will find them of interest, and I hope you will have time to get acquainted with them and with other resources of this University. Among Wisconsin dairy farmers and in the dairy industry too, you will find a warm welcome if you have time to do some traveling and visiting in the State.

The University facilities and staff are at your service, and all of us are looking forward to your stay in Wisconsin.

Sincerely yours,

C. A. ELVEHJEM, President

CALL FOR PAPERS FOR THE 1961 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION[1]

G. W. TRIMBERGER
Chairman, Program Committee, A.D.S.A.
Department of Animal Husbandry, Cornell University, Ithaca, New York

The 56th Annual Meeting of the American Dairy Science Association will be held June 12-14 at the University of Wisconsin, Madison. Members who wish to present papers must submit titles and abstracts not later than March 1. This deadline must be met to permit publication of titles with the complete program in the May issue and abstracts in the June issue of the JOURNAL. Last year all but four abstracts arrived on time. If a large number of papers are suggested for presentation, those that arrive late may be assigned to be read by title or rejected entirely.

Abstracts must contain no more than 200 words by actual count. Abstracts longer than 200 words cannot be accepted and will be returned to the author(s) by the Committee. An original (on bond paper) and three copies of each abstract must be typed double-spaced. The original and one copy should be mailed to the Chairman, another copy to the Vice-Chairman, and the fourth to the Secretary of the Section before which the paper is to be presented. Eventually, the original copy will be sent to the Editor for publication in the JOURNAL.

In the preparation of abstracts, the style and abbreviations of the JOURNAL OF DAIRY SCIENCE must be used. Please refer to abstracts in the June, 1960, issue of the JOURNAL for guidance. Only initials of authors should be used, except in unusual cases where it may be necessary to use the complete name. The title of the abstract should indicate clearly the nature of the research. It should not be repeated again in the text. The abstract should include, insofar as possible, the design and major results of the investigation. Only completed research should be reported. Brief, essential statistics will make the data more meaningful.

All members of the Association are entitled to present papers. Participation by members of the industry and by senior members of the Association is particularly encouraged. Attention is called to the Dairy Manufacturing Extension Section program, and members are urged to participate. This is a subsection of the Manufacturing Section for 1961. The competitive presentation of papers by graduate students, initiated in the Production Section in 1959 and in the Manufacturing Section in 1960, will be continued in both sections.

Papers submitted for the Annual Meeting should be confined to research that has not been reported previously. Abstracts of research articles accepted for publication by a scientific journal before the annual meeting are not acceptable, because this constitutes a duplication. The Program Committee favors the general policy that an individual present only one paper and that his name appear as author on no more than two. The Program Committee, together with the Association membership, wishes to stimulate vigorous, sound research and realizes that the above restrictions may penalize some members engaged in full-time research. Therefore, the Committee has liberalized this policy to the extent that a member can exceed these limits, but only if he or his department rates the abstracts in order of preference for oral presentation. If the total number of papers submitted by the membership is too great to include in the program, the Committee will assign some papers to be read by title only. In this event, consideration will be given to quality of abstract and research reported therein, number of abstracts per author and department, and importance of the research reported.

The Program Committee encourages continued improvement in quality of papers and in oral presentation. Careful design of experiments and proper interpretation of results are necessary prerequisites. Strict compliance with the instructions for preparation of abstracts will simplify the task of the Program Committee, and will improve the program. Careful editing of abstracts before submission is essential. In the past too many abstracts have been lacking in this respect.

All abstracts must be submitted on regular 8½ by 11-in. paper. The following form, with no capitals for the title, is correct and is in accordance with the form used in 1960:

> Utilization of carbohydrates posterior to the rumen-reticulum of the bovine. J. T. Huber and N. L. Jacobson, Iowa State University.

If the author lists an address for an experiment station other than the University, or a USDA research branch, or a commercial company, then the complete address should be provided, as presented in the following example from the 1960 program:

[1] The procedures for preparing abstracts will apply also to abstracts submitted for the Eastern, Southern and Western Divisional Meetings of the American Dairy Science Association. Editor-in-Chief.

A study of dye reduction methods as platform tests for the detection of antibiotics. Burdet Heinemann, Producers Creamery Co., Springfield, Missouri.

Preparation of mimeographed copies of pertinent data is desirable. At least 250 copies should be available for distribution. This can be supplemented by slides for projection on screens, provided the author can adhere to the assigned time of 12 to 14 min. for presentation of each paper.

Names and addresses of officers of sections to whom titles and abstracts should be sent are:

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(The Dairy Manufacturing Extension Section is a temporary subsection in the Manufacturing Section. R. B. Redfern, Dairy Extension, North Carolina State College, Raleigh, is chairman and W. S. Arbuckle, Department of Dairy Husbandry, University of Maryland, College Park, is secretary. All abstracts for papers in the Extension Section should be submitted through the regular channels of the Manufacturing Section, but should be identified for presentation at Extension Section meeting and a courtesy copy be sent to R. B. Redfern.)

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PROGRAM
ANNUAL MEETING OF SOUTHERN DIVISION OF THE AMERICAN DAIRY SCIENCE ASSOCIATION
JACKSON, MISSISSIPPI

February 6-8, 1961

Monday A.M.

DAIRY PRODUCTION

Presiding: J. B. FRYE, JR.

8:30–10:45 A.M.

Bamboo Room, King Edward Hotel

Lignin and other chemical constituents in Sudan grass and millet varieties. A. S. Achacoso, L. L. Rusoff, C. L. Mondart, Jr., and F. L. Bonner, Louisiana Agricultural Experiment Station.

Changes in digestibility of cool season forages during the spring grazing season. G. E. Hawkins and G. H. Rollins, Alabama Agricultural Experiment Station.

An unbreakable esophageal-fistula cannula for collecting forage samples by grazing cows. L. L. Rusoff and L. E. Foote, Louisiana Agricultural Experiment Station.

Influence of nitrogen fertilization and harvest date on the nutritive value of pelleted coastal Bermudagrass hay. R. R. Harris, W. B. Anthony, J. B. Starling, and C. A. Brogden, Auburn University.

The use of a small sample in vivo cellulose digestion technique for forage evaluation. J. W. Lusk, C. B. Browning, and J. T. Miles, Mississippi Agricultural Experiment Station.

11:00 A.M.–12:30 P.M.

General Convention Session, ASAW

Victory Room, Heidelberg Hotel

Monday P.M.

DAIRY PRODUCTION

Presiding: I. W. RUPEL

1:30–4:30 P.M.

Bamboo Room, King Edward Hotel

The total protein, casein, and butterfat content of colostrum milk. O. T. Stallcup and F. E. Reed, Arkansas Agricultural Experiment Station.

Gradual vs. abrupt weaning of dairy calves from a limited level of milk replacer. W. J. Miller and C. M. Clifton, University of Georgia.

Comparison of different calf starters for early weaned calves. L. J. Bush, G. V. Odell, and M. G. Yang, Oklahoma State University.

The importance of trace minerals in calf starter and milk replacer. E. W. Swanson and L. M. Carpenter, University of Tennessee.

Pasture vs. barn feeding for raising calves. B. F. Hollon and R. D. Mochrie, Coastal Plain Research Station, Willard, N. C.

An evaluation of the growth of dairy calves on a constant feeding regime in southern Louisiana. E. J. Stone, E. B. Morgan, and J. B. Frye, Jr., Louisiana Agricultural Experiment Station.

Frequency of grain feeding by mechanical means and growth rate response of dairy heifers. O. H. Horton and G. S. Nelson, University of Arkansas.

Factors concerned in hot weather effects on growth and feed efficiency of dairy heifers. J. E. Johnston, G. A. Hindery, D. H. Hill, and A. J. Guidry, Louisiana State University.

Monday A.M.

DAIRY MANUFACTURING

Presiding: H. C. OLSON

8:30–10:45 A.M.

Room 550, King Edward Hotel

Selective media for detection of spoilage organisms in dairy products. H. C. Olson, Oklahoma State University.

Coliform enumeration as an index of the sanitary quality of raw milk. R. W. Henningson, Clemson Agricultural College.

Coliform bacteria in Cottage Cheese. W. W. Overcast, J. D. Skean, and J. V. Britton, Jr., University of Tennessee.

Some results with the phosphatase test on butter. T. R. Freeman and J. L. Bucy, Kentucky Agricultural Experiment Station.

A microbiological study of Cottage Cheese made from frozen and daily transfer cultures. J. T. Cardwell, T. J. Ousley, and J. H. Martin, Mississippi State University.

11:00 A.M.–12:30 P.M.

General Convention Session, ASAW

Victory Room, Heidelberg Hotel

Monday P.M.

DAIRY MANUFACTURING
Presiding: J. T. CARDWELL

1:30–4:30 P.M.
Room 550, King Edward Hotel

Variations in the lactose and sodium chloride contents and the freezing point of certain reconstituted nonfat dry milks. E. W. Custer and J. T. Cardwell, Mississippi State University.

Rehydration of nonfat dry milk solids with home water supplies. A. V. Moore, Texas Agricultural Experiment Station.

Persistence of contamination in lactic cultures. H. C. Olson, Oklahoma State University.

Variations in cultures of geotrichum isolated from cream. E. R. Garrison and Mary Anne Holdar, University of Arkansas.

A comparison of the Kjeldahl, steam distillation, dye binding and formol titration methods for determining the protein content of milk. C. Vanderzant and A. H. Miah, Agricultural and Mechanical College of Texas.

The effect of chocolate milk powder mixtures on the accuracy of the Pennsylvania, Te Sa and Babcock-Gerber tests for fat in homogenized chocolate milk. J. T. Cardwell, Mississippi State University.

Relationship of surface tension to variations in composition and temperature of fluid dairy products. M. L. Peeples and H. J. Urban, Texas Technological College.

Monday P.M.

DAIRY EXTENSION
Presiding: C. G. CUSHMAN

1:30–5:00 P.M.
Room 546, King Edward Hotel

Mastitis

1:30–1:50 P.M.

Experiences in mastitis control in Florida. C. B. Plummer (DVM), University of Florida.

1:50–2:10 P.M.

Evaluation of mastitis control in Virginia. S. L. Kalison (DVM), Virginia Polytechnic Institute.

DHIA

2:10–2:30 P.M.

Care and accuracy of testing equipment used by DHIA associations. R. R. Rich and M. E. Senger, North Carolina State College.

2:30–2:50 P.M.

Use of quality codes in evaluating feeds in EDPM-DHIA records. R. S. Jones, Auburn University.

Break

2:50–3:00 P.M.

Artificial Insemination

3:00–3:20 P.M.

Responsibility of extension to artificial insemination programs. R. E. Burleson, Federal Extension Service, Washington, D. C.

3:20–3:40 P.M.

Responsibility of bull studs to artificial insemination programs. E. W. Neasham, Louisiana State University.

Panel Discussion

3:40–5:00 P.M.

Common problems in building dairy extension programs of work. Leader, V. L. Baldwin, Virginia Polytechnic Institute.

Monday P.M.

SOUTHERN DAIRY STUDENTS ASSOCIATION
Presiding: W. L. WEBB, Chairman SDSA

4:30 P.M.
Room 550, King Edward Hotel

7:00 P.M.
Dutch Dinner and Smoker
Time to be confirmed and place to be announced

Presiding: MARION MOORE, President
Mississippi State University Dairy Club

Tuesday A.M.

JOINT SESSION DAIRY PRODUCTION, DAIRY MANUFACTURING, AND DAIRY EXTENSION
Presiding: T. R. FREEMAN

8:30–9:45 A.M.
Bamboo Room, King Edward Hotel

The influence of certain environmental factors on the fat and solids-not-fat production of Jersey cows. J. C. Johnson, Jr., and R. L. Givens, Georgia Coastal Plain Experiment Station, Tifton.

Certain factors affecting the solids-not-fat (SNF) and total solids (TS) in milk. O. T. Fosgate, University of Georgia.

The effect of feed and nutritional level of the cow on the development of rancidity in milk. R. Y. Cannon and G. H. Rollins, Alabama Agricultural Experiment Station.

The effect of feeding sorghum silage on the lipolytic activity in milk. P. E. Johnson and R. L. Von Gunten, Oklahoma State University.

Daily variations in lipolytic activity in individual cows milk. P. E. Johnson and R. L. Von Gunten, Oklahoma State University.

9:45–11:00 A.M.

Southern Division, ADSA, Business Meeting
Bamboo Room, King Edward Hotel

11:00 A.M.–12:30 P.M.
General Convention Session, ASAW
Victory Room, Heidelberg Hotel

Tuesday P.M.

JOINT SESSION DAIRY PRODUCTION, DAIRY MANUFACTURING, AND DAIRY EXTENSION

Presiding: W. S. GRIFFITH

1:30–4:30 P.M.

Bamboo Room, King Edward Hotel

A critical appraisal of the central processing program for DHIA records. W. S. Griffith, Virginia Polytechnic Institute.

Nutrient composition and digestibility of corn silage as affected by fertilizer rate and plant spacing. R. A. Alexander, G. A. Barden, J. F. Hentges, Jr., J. T. McCall, and W. K. Robertson, Florida Agricultural Experiment Station.

Observations on the digestibility of sweet sorghum silage ensiled at four stages of maturity. D. S. Ramsay. J. W. Lusk, and J. T. Miles, Mississippi Agricultural Experiment Station.

The relative feeding value of coastal bermuda grass silage and corn silage for lactating dairy cows. J. C. Johnson, Jr., F. E. Knox, and B. L. Southwell, Georgia Coastal Plain Experiment Station, Tifton.

The use of two levels of distiller's grains and ground snap corn as preservatives for wheat silage. M. E. McCullough and L. R. Sisk, Georgia Experiment Station, Experiment, Ga.

The performance of lactating dairy cattle when fed millet silages harvested at two stages of growth with and without ground snapped corn as an additive. E. B. Morgan and H. Dewitt Ellzey, Southeast Louisiana Dairy and Pasture Experiment Station, Franklinton.

Genetic and environmental differences among dairy herds. N. R. Thompson, V. L. Baldwin, and G. C. Graf, Virginia Agricultural Experiment Station.

6:30 P.M.

Annual Banquet and Honors Program
Speaker: R. E. HODGSON, President ADSA

Tuesday A.M.

SOUTHERN DAIRY STUDENTS ASSOCIATION

Presiding: JOE LINEWEAVER, Vice Chairman SDSA

8:30–10:45 A.M.
Room 550, King Edward Hotel

11:00 A.M.–12:30 P.M.
General Convention Session, ASAW
Victory Room, Heidelberg Hotel

Tuesday P.M.

SOUTHERN DAIRY STUDENTS ASSOCIATION

Presiding: W. L. WEBB, Chairman SDSA

1:30 P.M.

Room 550, King Edward Hotel

6:30 P.M.

Annual Banquet and Honors Program
Southern Division, ADSA
Speaker, R. E. HODGSON, President ADSA

Wednesday A.M.

JOINT SESSION DAIRY PRODUCTION, DAIRY MANUFACTURING, AND DAIRY EXTENSION

Presiding: O. T. STALLCUP

8:30–11:00 A.M.

Bamboo Room, King Edward Hotel

The effects of feeding protamone to bulls on semen production libido, and physiological responses. T. E. Patrick, J. D. Roussel, H. C. Kellgren, and J. O. Shelwick, Louisiana State University.

Effects of nitrogen and other gases on livability and fertility of frozen bovine spermatozoa. J. D. Roussel, T. E. Patrick, and H. C. Kellgren, Louisiana State University.

The effect of vitamin B_2 deficieney in the gonads of male rats. N. Pati, Agricultural and Technical College of North Carolina, Greensboro.

Livestock improvement in the Middle East. E. M. Harmon, Hendersonville, N. C.

Wednesday A.M.

SOUTHERN DAIRY STUDENTS ASSOCIATION

New Officers in Charge

8:30 A.M.

Room 550, King Edward Hotel

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